Federation Proceedings

VOLUME 11 *March 1952* NUMBER 1, PART I

THIRTY-SIXTH ANNUAL MEETING

NEW YORK CITY, APRIL 14–18, 1952

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THE AMERICAN PHYSIOLOGICAL SOCIETY

Sixty-fourth Annual Meeting

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*An asterisk * following an author's name indicates "by invitation"*

Phosphorus metabolism and function in the nervous system. L. G. ABOOD AND R. W. GERARD. *Dept. of Physiology, Univ. of Chicago, Chicago, Ill.*

Brains of pure-bred mice strains susceptible or non-susceptible to sound-induced seizures (courtesy of Dr. B. Ginsburg), were compared. Susceptible brains were lighter, per body weight, than normals. Essentially no differences were found in glycolytic and oxidative rates and enzymes, but P metabolism was defective in the audiogenic animals during the susceptible period. ATP-ase activity was about 25% below normal, alkaline phosphatase 5% low (statistically significant), the P/O ratios definitely below 2.0 rather than well above, and the turnover of labile phosphorylated intermediates (P^{32}) definitely slowed. The content of phosphorylated intermediates, except for a slightly higher ATP and CrP, and the turnover of phospholipids, nucleins and phosphoproteins were normal. Liver glycogen was about half normal in the susceptibles. Parasympathomimetic drugs inhibit the oxidative phosphorylation of rat brain and spinal cord *in vitro;* 10^{-4}M physostigmine or prostigmine over 50% and 5×10^{-4}M DFP completely, with even 5×10^{-6}M giving 20%. *In vivo* DFP, $2 \times LD_{50}$, produced a third inhibition; the other drugs a fourth or less. The appearance of convulsions with disturbed phosphorylation—in audiogenic strains and in DFP poisoning, as well as in poisoning by cyanide and other metabolism inhibitors and in hypoxia and hypoglycemia—is suggestive of a significant relationship beyond the obvious one of energy-rich P being split to support activity. Perhaps, as in muscle, over-activity accompanies lowered ~P. (Supported by ONR contract.)

A method for producing shock following experimental myocardial infarction in the closed-chest dog. CLARENCE M. AGRESS,* MARVIN J. ROSENBURG,* MAXWELL J. BINDER,* ABRAHAM SCHNEIDERMAN,* HOWARD I. JACOBS* AND W. G. CLARK. *Cardiovascular Lab., Veterans Administration Center, Los Angeles, Calif.*

A double-lumen metal catheter with an inflatable rubber balloon near the tip, and with a sliding inner bore, has been devised for the injection of plastic microspheres, in diameters up to 600 micra, suspended in acacia of the proper specific gravity. The catheter is passed down the left internal carotid artery to the aortic valves, as previously described for the open-chest dog (*Am. J. Physiol.* 166: 603, 1951). The balloon, constructed to withstand high pressures, is hydraulically inflated using physiological saline. The number, general distribution and the percentage of the microspheres entering the heart, as compared to the other major organs, has been studied, using I^{131} labeled microspheres. Studies to date show that approximately 70% of the injected dose enters the coronary circulation. The remainder is filtered out largely in the skin and muscles, and only small amounts enter the kidney, brain and other organs. Graded myocardial injury can be produced by this technique. In the majority of instances, percentage decreases in blood pressure comparable to shock levels in humans can be maintained for several hours before death occurs. The data suggest that the bead size is critical for the production of the blood pressure fall. A total of 45 dogs has been used. The technique described avoids the factors of trauma and hemorrhage inherent in opening the chest, and with experience, has proved most satisfactory. (Supported by the L. A. Heart Association.)

The contribution of vascular 'hysteresis' to blood pooling. ROBERT S. ALEXANDER. *Dept. of Physiology, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

Previous investigations of splanchnic blood pooling produced by acute portal hypertension have shown that relatively small volumes of blood are pooled by simple elastic distension of the veins, while much larger volumes are pooled by some process which shows relatively slow equilibration with portal pressure. The latter mechanism has been further elucidated by studying venous blood flow and pressure relationships in the intact splanchnic bed or in intestinal loops of anesthetized dogs. In addition, recordings have been made of pressure changes accompanying injections into intestinal veins immediately after occlusion of the

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blood supply. The pressure-volume relationships observed in these experiments present further evidence that increase in venous pressure produces a progressive pooling of blood due to a gradual hysteresis-like distension of the vascular bed. Study of this mechanism during adrenaline infusion suggests that the smaller vessels make a significant contribution to this 'hysteresis' pooling.

Adaptation of radiothermic wave band. FREDERICK M. ALLEN. *City Hosp., Welfare Island, N. Y.*

A method is described whereby electromagnetic waves in the radio frequency range can be employed biologically with better localization and less disturbing heat effects. Experimental observations in several animal species and possible clinical applications are illustrated.

Response of rat temperature and respiratory rate to thermal stress, magnesium chloride and tetrazolium salts. SHANNON C. ALLEN AND VICTOR E. HALL. *Dept. of Physiology, Stanford Univ. School of Medicine, Stanford, Calif.*

On the hypothesis that CNS thermoregulatory centers respond to thermal stress with increased metabolic activity, tetrazolium salts were injected into unanesthetized, restrained, mature, male Sprague-Dawley rats. It was hoped that such centers might be located by differential deposition of colored formazans. It was found that the rat does not respond to increased environmental temperature by panting nor does it maintain normal rectal temperature at environmental temperatures beyond 34°C. above which rectal temperature rises sharply and almost linearly with rising environmental temperatures. Death occurs at rectal temperatures between 44 and 45°C. The only apparent heat defense mechanisms utilized are peripheral vasodilatation, and increased salivation accompanied by vigorous and frequent head washing with the forepaws. Intraperitoneal injections of isotonic $MgCl_2$ in concentrations up to 1.5 mM/kg. failed to induce panting at room temperature but did cause a marked drop in rectal temperature as did i.p. doses of 2,3,5-triphenyl tetrazolium chloride as low as 5.0 mg/kg. and Neotetrazolium, either i.p. or i.v., in doses as low as 1.0 mg/kg. Formazan deposition was confined to the peritoneum and visceral organs in the case of both tetrazolium salts even with doses as high as 20 mg/kg. It is concluded that the rat is not suitable for investigations of response to high thermal stress and that the tetrazolium salts used do not reach the brain at non-toxic dose levels.

Measurement of plasma volume in the dog with high concentrations of T-1824. THOMAS H. ALLEN AND MAGNUS I. GREGERSEN. *Dept. of Physiology, College of Physicians and Surgeons, Columbia Univ., New York City.*

Paired successive estimates of plasma volume in normal dogs indicate statistically that the volume distribution is independent of the amount of T-1824 up to concentrations of more than 1 mg/ml. Greater concentrations, in the course of mixing in the blood of a dog, produce hemolysis; the extent of which can be detected by use of paper pulp extraction. This procedure separates the T-1824 from the hemoglobin, and the plasma concentration of each material can then be measured photometrically without interference. Half of the circulating red cells are lyzed by amounts of T-1824, which ultimately reach a concentration of 1.6 mg/ml. of plasma. The hemolytic effect of T-1824 occurs *in vitro* as well as *in vivo*. However, thrice-washed blood cells in saline are 50% destroyed by only 0.02 mg. of T-1824/ml., which corresponds to the concentration of free dye in the plasma, when the total dye is 3.2 mg/ml. The human red cell is extremely resistant and either in plasma or in saline is not affected by 4 mg. T-1824 ml. The species difference in resistance to T-1824 suggests that the red cell membranes contain proteins of differing composition.

Activity of electroplax of electric eel. MARIO ALTAMIRANO-ORREGO,* CHRISTOPHER W. COATES,* HARRY GRUNDFEST, DAVID NACHMANSOHN AND IRWIN B. WILSON.* *Dept. of Neurology, College of Physicians and Surgeons, Columbia Univ., and New York Zoological Society, New York City.*

Electrical activity has been studied in the whole eel, in surviving perfused slices, and in single electroplax, as part of an attack on correlations of electrical and chemical processes in this highly favorable material. Single shock nerve stimulation is relatively ineffective, but a second stimulus soon thereafter to the nerve produces activation of the electroplax. The facilitation curve rises slowly, after a delay, reaching its maximum at 15 msec.; decaying slowly (half-time about 60 msec.); remaining clearly measurable for more than 100 msec. In slices, build up to slow repetitive stimulation demonstrates facilitation persistent for more than 1 sec. The facilitation curve is temperature dependent, the peak optimal at about 22°C. Its form is altered by inhibitors of acetylcholinesterase. This class of compound also blocks activity. Unit responses to nerve stimulation are abolished by curare, but direct stimulation remains effective. The resting potential of single units is at least 40 mv.

A comparison of rates of incorporation of iron and of the α-carbon atom of glycine into hemoglobin. KURT I. ALTMAN, THOMAS R. NOONAN, SANFORD C. SPRARAGEN AND KURT SALOMON (introduced by HENRY A. BLAIR). *Depts. of Radiation Biology and Biochemistry, Univ. of*

Rochester, School of Medicine and Dentistry, Rochester, N. Y.

The rates of incorporation of the α-carbon atom of glycine into hemin and globin have been shown to vary independently under such diverse experimental conditions as x-radiation and starvation (NOONAN, T. R., *et al. Federation Proc.* 8: 119, 1949; RICHMOND, J. E. *et al. J. Biol. Chem.* 190: 817, 1951). It seemed of interest, therefore, to determine whether the rate of incorporation of iron into hemin parallels the rate of incorporation of the α-carbon atom of glycine into protoporphyrin. For this purpose, young adult rats were injected intraperitoneally with α-C^{14}-glycine (3 μc.) and with Fe^{59} (0.5 μc.) and were sacrificed at varying time intervals. For each time interval the pooled erythrocytes from 2 or 3 rats were used to prepare crystalline hemoglobin. An aliquot of this hemoglobin preparation was ashed and used to determine Fe^{59} concentration, while the remainder was used to prepare dimethylprotoporphyrin and globin for the determination of C^{14} activity. The data indicate that the ratio of Fe^{59}/protoporphyrin-C^{14} activities remains nearly constant in the normal animal over a period of 48 hours after the injection of the isotopes. The greatest increase in isotope concentration in iron as well as in protoporphyrin and globin occurs during the time interval between 24 and 48 hours after injection of the isotopically labeled materials. (This work was performed under contract with the U. S. Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, N. Y.)

Mechanism of generalized motor reactions in cats under chloralose anesthesia. E. C. ALVORD, JR. AND M. G. F. FUORTES (introduced by D. McK. RIOCH). *Army Med. Service Grad. School, Washington, D. C.*

Although sustained reflex actions are of major physiological importance, under anesthesia long-lasting reactions are impaired, and only brief reactions to sudden stimuli remain. Under chloralose anesthesia, the reactions to sudden stimuli are not only highly concentrated in time, but also spread widely through the spinal cord and brain stem, so that generalized jerks of the musculature result. This generalized response follows the stimulus after a variable delay, ranging in most cases between 10–40 msec. In general the delay is shorter when the afferent impulses enter the c.n.s. at higher levels of the spinal cord or brain stem. The reaction to a particular stimulus may take place simultaneously in widely separated segments of the cord. The above time characteristics suggest that the development of sufficient c.e.s., not conduction along central nerve pathways, is the principal cause of the delay. Indeed, some mechanism other than nerve conduction may be responsible for the spread, since excitation has been observed to cross a complete spinal transection. Below ipsilateral spinal hemisection or contralateral hemidecerebration or hemidecortication, a stronger stimulus is needed to evoke generalized responses, indicating that supraspinal centers influence its development.

Organization in somatosensory systems. V. E. AMASSIAN (introduced by A. A. WARD, JR.). *Dept. of Physiology and Biophysics, Univ. of Washington School of Medicine, Seattle.*

In addition to the familiar responses in somatosensory areas I and II, longer latency (22–28 msec.) responses occurred maximally in the somesthetic association area in the lateral gyrus a few millimeters behind the ansate sulcus in cats under chloralose-tubocurarine. Stimulation of both cutaneous and deep nerves from either forelimb or hindlimb on either side was effective. Maximal foci were almost identical for all four limbs but response shapes differed. Responses from stimulation of arm and leg subdivisions of somatosensory area I were similar in location but differed in shape. Click and visual responses were also observed in this area. Complete blocking interaction (facilitation at longer test intervals) occurred between any pair of afferent inputs mentioned. Microelectrode recordings were made of single soma activity evoked by tactile or nerve stimulation in A-association cortex; S-somatosensory areas I and II; VP-thalamic ventralis posterior nucleus; R-pons, tegmentum and medial thalamic regions. (Significantly, some cells commence firing in the somatosensory areas under either barbiturate or chloralose anesthesia even before the peak of the primary positive response suggesting that they are partly responsible for the latter.) Any unit isolated in A or R, if fired from one limb, was fired by all, although in A-units latency and rhythm of firing sometimes differed. The receptive field for VP and S units was smaller and increase of latency with reduction of number of spikes/discharge occurred when the margin of the unit's receptive field was stimulated. R activity (latency 7–14 msec.) reflects extreme subcortical afferent convergence (confirming MAGOUN *et al.*) but differs in organization from A activity.

Calcium metabolism of growing and mature bone. JAMES S. ARNOLD (introduced by GEORGE SAYERS). *Radiobiology Lab., Univ. of Utah College of Medicine, Salt Lake City.*

A radioautographic study of the Ca-45 distribution in the bones of growing and mature rats and rabbits has been made. Animals were given Ca-45 intravenously in tracer amounts, and sacrificed over a period of 3 weeks. Radioautograms were made from 5–10 micron sections of the undecalcified bones. A clear picture of the pattern of bone growth on a histologic scale is demonstrated. Ion

exchange experiments on various areas of bone from the animals have been performed. The degree of *in vitro* exchangeability of the Ca-45 deposited *in vivo* is determined. The relative importance of ion exchange on the surface of bone salt crystals in growing and mature bone is evaluated. Ethylene diamine hydrolysis and extraction of bones gives information about the relative amount of Ca-45 combined with the organic matrix at various time intervals after deposition.

Dilating action of anoxia on pulmonary vessels. D. M. AVIADO, JR., A. CERLETTI,* J. ALANIS,* P. H. BULLE* AND C. F. SCHMIDT. *Lab. of Pharmacology, Univ. of Pennsylvania, Philadelphia.*

Dogs under morphine and chloralose that were subjected to the spontaneous inhalation of 5 or 10% oxygen responded in the following manner: *a*) Pressure of the cannulated lobar artery increased by about 30 mm. H_2O for an arterial blood O_2 saturation of 60%. *b*) This pulmonary hypertension was caused chiefly by an anoxic rise in pulmonary flow. When the flow to the lung (one side) was kept constant by pumping the mixed venous blood from the right auricle to the pulmonary artery, the corresponding pulmonary arterial pressure decreased while the pressure of the other side that is supplied by its own heart increased, during anoxia. *c*) An isolated perfused lung showed vasodilatation when oxygen content of either the ventilating air or the perfusing blood was reduced. *d*) The observed pulmonary hypertension in anoxic intact dogs was the combination of this local vasodilatation and of the increased pulmonary flow. The latter was elicited by the anoxic stimulation of carotid and aortic chemoreceptors and by the liberation of epinephrine. *e*) The net effect of the above responses was reflected in the radioactive measurement of erythrocytes tagged with phosphorus^{32} by means of a beta counter applied to the surface of the lung. This estimation of capillary blood volume in the lungs was observed to increase by about 10% during anoxia. All these results in dogs are different from the reports that low oxygen content constricts the perfused pulmonary vessels of cats.

Relationships between bulbar respiratory, vasomotor, and somatic facilitatory and inhibitory areas. L. M. N. BACH. *Dept. of Physiology, Tulane Univ. School of Medicine, New Orleans, La.*

A series of 43 cats under various anesthetics were investigated using stereotaxically oriented bipolar electrodes with 5V 60 cps stimuli applied to various points in the medulla and lower pons. Recordings were made of the recurrently elicited kneejerk, respiration and blood pressure. The nature of the anesthetic was not found to affect the results significantly. The most frequent response combination (45%) consisted of a vasopressor response and kneejerk inhibition, the next two most frequent combinations being slight expiratory apnea with kneejerk inhibition and vasodepression with kneejerk inhibition. The three most common combinations of changes of all three functions consisted of a vasopressor response, slight expiratory apnea, and kneejerk inhibition (14%) followed by a combination consisting of vasodepression, slight expiratory apnea, and kneejerk inhibition, this in turn being followed by the combination of vasodepression, midpositional apnea, and kneejerk inhibition. While brainstem vasomotor pressor point and kneejerk facilitatory point distributions were similar histologically they apparently were not identical since there was poor correlation of facilitatory effects with vasopressor effects. At the level of the facial nucleus in the lower pons there was a distinct grouping of points having a dorsal and ventral distribution in the medial reticular formation which when stimulated produced kneejerk facilitation, forced expiration or inspiration, and vasopressor effects while ventral and lateral to this area were grouped points which when stimulated produced kneejerk inhibition, apneas and vasodepression. Kneejerk inhibitory points were preponderant especially in the caudal medullary areas.

Color of ultraviolet light. A. BACHEM. *Dept. of Physiology, Univ. of Illinois, Chicago Professional Colleges, Chicago, Ill.*

Experimental studies on ultraviolet color vision of young subjects and aphakics by means of a Kromayer lamp, a Noviol filter and a quartz monochromator verify Helmholtz's statements concerning the reversal of the spectrum towards blue and the decreasing saturation towards shorter wave lengths. Both effects become noticeable at or below 391 mμ. Spectrometric examinations of the fluorescence of the eye media refute Helmholtz's assumption that the retinal fluorescence is responsible for the lavender-gray of the ultraviolet light. Maximal bluish-white fluorescence occurs near 391 mμ and decreases rapidly towards shorter wave lengths. The author assumes that the fundamental color of short wave light is indigo-blue. This blue is discolored near its center into violet either by a secondary maximum of Helmholtz's red curve or by a red or purple modulator in Granit's terminology. Spectroscopic observations of protanopes and deuteranopes and reports on tritanopes give evidence for a red component of violet sensation. Further proof is offered by monocular adaptation to red and blue light (contralaterally controlled), which disposes of one or the other of the components of violet. The known facts of spectral saturation and hue-

discrimination are in agreement with this assumption. Also the Brücke-phenomenon, as far as the shift from violet to blue with increasing illumination is concerned, finds its explanation on this basis. The progressive lack of saturation towards shortest wave lengths may be due to the superposition of the short wave ends of Helmholtz's 3 color-curves or Granit's dominators and modulators.

Fechner's law as a dose-action law. A. Bachem. *Dept. of Physiology, Univ. of Illinois, Chicago Professional Colleges, Chicago.*

Fechner's law, based upon the assumption of a constant $\Delta i/i$, was originally established as a straight line relationship between the logarithm of the stimulus and the sensation. Since Weber's quotient was recognized as variable, Fechner's semilogarithmic straight line was replaced by a sigmoid. Its point of flexion represents the minimum of $\Delta i/i$. Houstoun has shown that the reciprocal of Weber's quotient, $i/\Delta i$, fits the Gaussian curve of error and thus represents the probability distribution of sensory response. In contrast to Hecht, Houstoun, Hoagland, who attribute the probability distribution to a single factor (excitatory process, nerve fibers, synapses) the author makes the summation of many factors, or the superposition of several probability curves, responsible for the broad minimum of Weber's quotient and the relatively straight sigmoid of the integral curves. Simple responses approach the non-threshold, fading-out reaction of single hit- or holistic-mechanisms. The author's quantitative studies of muscular and glandular response to direct stimulation demonstrate reactions of this type. The complex mechanisms of sensation and homeostasis lead to drawn-out sigmoids with absolute thresholds. In the particular case of sensation, the all-or-none response of the nerve fiber and synaptic resistance account for the threshold; the excitatory process, the receptors, the nerve impulses and synaptic summation (subliminal fringe, occlusion) produce the relative linearity; the maximal response of these mechanisms causes the fading-out effect. The corrected Fechner law thus represents a complex dose-action relationship.

Protective action of Dibenamine against the fatal outcome of hemorrhagic and traumatic shock in rats. Silvio Baež, Benjamin W. Zweifach and Ephraim Shorr. *Dept. of Medicine, Cornell Univ. Med. College, and The New York Hosp., New York City.*

Rats subjected to a standardized type of hemorrhagic shock, using a self-infusion reservoir, and to trauma in the Noble-Collip drum, were given various doses of a Dibenamine derivative (S.K.F. 688A). The administration of 20 gamma Dibenamine/100 gm. body weight, 30–60 minutes prior to bleeding, resulted in 100% survival as compared with 37.5% in normal controls bled in an identical manner. The administration of 5 or 10 gamma Dibenamine was almost as effective. Dibenamine protected animals could be maintained at drastic levels of hypotension (35 mm. Hg) with a minimum uptake of blood from the reservoir in contrast to controls which began to take up blood as early as 20 min. after reaching maximal blood loss. Infusion of all the blood withdrawn after 4 hours of hypotension restored the blood pressure of Dibenamine treated rats to normal levels almost immediately. Not only were Dibenamine treated rats found to be resistant to the deleterious effects of hemorrhagic hypotension, but they could be recovered by the infusion of as little as 50% of the total blood withdrawn. A similar protective action was noted in rats subjected to 700 revolutions in the Noble-Collip drum. Dibenamine treated animals showed a 92% survival as contrasted with a 28% survival for control rats. The effects of nephrectomy and adrenalectomy on the capacity of Dibenamine treated animals to withstand drum trauma and hemorrhage were also investigated.

Enzyme assays in nucleated and non-nucleated erythrocytes. Alice S. Baker and F. R. Hunter (introduced by E. Newton Harvey). *Dept. of Physiology, Florida State Univ., Tallahassee.*

It is known that nucleated erythrocytes consume oxygen at a higher rate than mammalian erythrocytes. To investigate this basic metabolic difference, a comparison of the activity of various enzymes is being made. As initial experiments, the activities of some enzymes in the Krebs cycle as well as those involved in the terminal steps of glycolysis were investigated. These included succinic, malic and lactic dehydrogenases. A reaction between added ascorbate and hemoglobin made it impossible to interpret the data obtained from an attempt to measure the activity of cytochrome oxidase. Homogenates of human and chicken erythrocytes prepared by alternate freezing and thawing were used in these studies. Standard techniques for enzyme assays involving the use of a Warburg apparatus were employed. All determinations were made at 37° C. The data indicate a higher activity in chicken erythrocyte homogenates for the three systems studied. The microliters of oxygen consumed per 100 mg. dry weight of cells per hour for chicken and human erythrocytes respectively are: succinic dehydrogenase 52 and 4; malic dehydrogenase 58 and 8, and lactic dehydrogenase 43 and 11. These experiments suggest that the low rate of oxygen consumption of human erythrocytes may result from a low level of activity of some of the enzyme systems in these

cells. Whether or not this is a consequence of a small amount of enzyme present, presence of inhibitors or some other factor such as instability of the enzymes in homogenates cannot at present be stated. (This work was aided by a contract with the Office of Naval Research, NR 120-077.)

Influence of cortisone and ACTH on capillary permeability in rats under subnutritional conditions. THEODORE BALOURDAS* AND ROBERT CHAMBERS. *Dept. of Biology, New York Univ., New York City.*

In an attempt to ameliorate the abnormal manifestations of the capillary vessels under subnutritional conditions, treatment has been done with cortisone and ACTH on rats under deficient nourishment. For this investigation the mesoappendix technique published by Chambers and Zweifach was used (*Am. J. Anat.* 75: 173, 1944). This bioassay consisted of direct visualization of the capillary vessels of the rat mesoappendix and of the blood circulation within them. The exteriorized mesoappendix was kept moist by a drip 1% ash-free gelatin Ringer's solution (37–38° C.) at pH 7.4. The rats were under subnutritional diets (protein-free diet, dextrose diet and other subnutritional diets) over a period of 2–12 weeks. The observation of capillary vessels of the exposed mesoappendix at various stages of the substarvation and at various times showed: *1*) increased epinephrine sensitivity from the normal 1:4 ml. to 1:5000 ml. and more; *2*) increased permeability of the capillary walls, indicated by leakage of intravenously injected water blue; *3*) slowing of the blood flow, frequent stasis, petechial hemorrhages, dilatation, etc. These findings have been published (BALOURDAS, THEODORE. *Bull. N. Y. Acad. Med.* 27: 386, 1951). These starved rats were given cortisone and ACTH administered intermuscularly. The cortisone was injected in dosages of 1–2 mg. daily for 5–10 days; ACTH, in 50–100 γ (gamma) daily for 2–10 days until the microscopic examination. The starved animals treated with these hormones exhibited a striking reduction of capillary leakage, a lowering of the epinephrine sensitivity, a betterment of the blood flow without stasis and petechial hemorrhages.

Cardiac output (direct Fick) during graded exercise of normal dogs, dogs with decreased cardiac competence, and with congestive failure. A. C. BARGER, V. RICHARDS,* J. METCALFE* AND B. GÜNTHER.* *Dept. of Physiology, Harvard Med. School, Boston, Mass.*

The 'resting' cardiac output of normal, trained dogs standing on the treadmill before exercise varied from 100–240 ml/kg/min. Cardiac outputs during a second rest period after 3 minutes of walking on the level were 100–200 (mean 156) ml/kg/min. The higher and more variable initial values appear to be due to excitement. In this initial period of excitement CO had no regular relationship to oxygen consumption. Using the lower and less variable resting values for comparison, graded exercise increased CO progressively to as high as 560 ml/kg/min.—a 4- to 5-fold increase over resting. The curve relating the normal dog's CO to oxygen consumption during exercise has a slope remarkably similar to that of man and horse. After tricuspid insufficiency CO fell in the normal range (though on the low side), and increased 2- or 3-fold during moderate exercise. With heavy exercise CO leveled off at values about half the normal maximum. The resting CO of dogs with mild pulmonary stenosis and mild tricuspid insufficiency similarly fell in the low, normal range, and the maximum output was about half the normal maximum. Combined tricupsid insufficiency (severe) and moderate to severe pulmonary stenosis produced overt congestive failure, reduced resting cardiac output to between 85 and 140 ml/kg/min., and abolished almost entirely the rise of CO during exercise.

A caloric technique for measuring blood flow in feline bladder. CARL M. BARLOW* AND WALTER S. ROOT. *Dept. of Physiology, College of Physicians and Surgeons, Columbia Univ., New York City.*

In cats under Nembutal anesthesia the ureters were ligated, and the bladders were exteriorized. Evaporation and heat loss were reduced by surrounding the organ studied and the controls, consisting of a rubber balloon or a freshly excised cat's bladder, with rubber dam and cotton swathing. The increase in the temperature of a measured volume of saline (20°–25° C.) introduced through the cannulated urethra and remaining in the suspended viscus for a definite period of time was determined. At constant intravesical volumes the caloric exchange showed a linear relation to time when measured for separate periods of 1, 2, 3, 4, and 5 minutes. The difference between the caloric exchange estimated under these conditions and that observed in controls containing the same volume of fluid and placed adjacent to the organ studied was assumed to represent the heat exchange produced by the blood flow. This was tested by clamping the aorta or by inducing cardiac arrest. Either of these procedures caused the heat exchange to decrease to the level shown by the excised bladder or rubber balloon. When the bladder volume was increased the caloric exchange increased in a linear manner until the intravesical volume was sufficiently great to produce an abrupt rise in the resting intravesical tension. Above this point a marked decrease in caloric exchange was noted. Further increments

(10–30 cc.) in volume produced hemorrhage into the bladder lumen, edema of the wall, and disruption of the mucosa.

Relationship of exposure to cold and radiation mortality. J. C. D. BARLOW* AND E. A. SELLERS. *Dept. of Physiology, Univ. of Toronto, Toronto, Canada.*

The effect of exposure to cold on the mortality rate after acute subletha! radiation was studied in adult Wistar rats. Exposure to cold and radiation were arranged according to the following schedule: *1*) exposed to normal environmental temperatures, irradiated and returned to normal temperature; *2*) exposed to normal environmental temperatures, irradiated, and exposed to a cold environment (1–2°C.); *3*) exposed to cold environment for at least 6 weeks, irradiated, and returned to cold; *4*) exposed to cold environment, irradiated, and returned to a normal temperature. Rats were irradiated in randomly distributed groups and the radiation was carried out as rapidly as possible using a 200 kv. 15 ma therapeutic x-ray machine with suitable filtration. Exposure to cold in addition to the radiation increased the mortality rate. When rats were acclimatized to cold, and returned to a normal environment subsequent to radiation, the mortality rate (28 days) was consistently less than in other groups. This indicates that acclimatization to cold increased the resistance of the animal to radiation as well as to cold. The long-term mortality of surviving rats has not been investigated, and the degree of protection afforded by acclimatization to cold requires further study. (This work was supported by the Defence Research Board of Canada.)

Oxygen delivery from erythrocytes polarographically determined. J. PERCY BAUMBERGER AND GABOR MARKUS.* *Physiology Dept., Stanford Univ. Med. School, Stanford, Calif.*

The relative rate of transfer of oxygen from the erythrocyte to a region of zero oxygen tension has been determined from the oxygen diffusion-current at a dropping-mercury electrode in a polarographic circuit. This transfer of oxygen may be comparable to the delivery of oxygen taking place in the tissues. The following factors affect the rate: pH, temperature and % saturation. The diffusion-current is greater for blood than for plasma at the same oxygen tension. That this difference is due to the excess of oxygen delivered to the electrode from the erythrocytes in the immediate neighborhood, is shown by the following observations. There is a linear relation between current and % oxygen saturation for any given blood sample. The oxygen-diffusion-current is a linear function of the red cell count when other factors are kept constant. Care must be taken to measure the current at the plateau of the polarographic oxygen wave rather than at a fixed applied voltage, as the cells modify the conductivity and, as a consequence, the plateau is shifted to more negative applied voltages as the cell count increases. The oxygen delivery rate represents a functional measurement which may be useful in characterizing any particular blood sample with respect to this most important aspect.

Hypophyseal involvement in response to O_2 at high pressure. JOHN W. BEAN AND PAUL JOHNSON.* *Dept. of Physiology, Univ. of Michigan, Ann Arbor.*

The demonstration of adrenal reaction in animals exposed to O_2 at high pressure reported earlier (J. W. B. *Federation Proc.* April 1951) called for a reconsideration of the hypophysis in O_2 poisoning. Young adult albino rats, hypophysectomized and controls, were repeatedly exposed to O_2 at from 70- to 90-lb. short periods (3–20 minutes) once or twice daily for several days or weeks. CO_2 was continuously removed from the chamber by soda lime; temperature was held at approximately that of the room and decompression was such as to avoid bubble formation. Although there was some individual variation, the data showed that hypophysectomy definitely delayed the onset and decreased the severity of the acute neuromuscular response and convulsive seizures induced by OHP. The severity of chronic motor paralysis, commonly induced by repeated exposure to OHP, was reduced or their occurrence completely eliminated; dyspnea was much less severe and the mortality diminished. Hypophysectomy provided marked protection against pulmonary damage by OHP. Lungs of controls succumbing to OHP commonly showed severe damage, hemorrhage, congestion and a liver-like appearance in parts or all of the lung, which frequently sank in H_2O whereas those of hypophysectomized animals sacrificed under anesthesia were much less severely affected, some remaining entirely clear despite a much more rigorous exposure to OHP. Lowered metabolism may explain in part the delayed onset of acute neuromuscular reactions in the hypophysectomized animals, but their greater resistance to chronic effects of O_2 poisoning, such as motor paralysis and lung damage, are not satisfactorily explained on this basis.

RBC ergothioneine levels in experimentally produced diabetes in rats. CLARISSA H. BEATTY. *Depts. of Biochemistry and Physiology, Univ. of Oregon Med. School, Portland.*

There has been considerable interest recently in the relationship of SH compounds to diabetes (*Am. J. M. Sc.* 219: 353, 1950; *J. Lab. & Clin. Med.*

37: 199, 1951) has reported abnormally high RBC ergothioneine levels in a few cases of diabetes mellitus. We have investigated RBC ergothioneine concentrations in rats made diabetic by experimental means. Because young rats were found to have significantly lower RBC ergothioneine levels than mature animals, the effect of diabetes was studied in mature rats whose ergothioneine levels were stabilized. Repeat determinations 1–2 weeks apart showed an average difference of only −0.15 mg. % RBC ergothioneine (t = <1.0, n = 15). A 3-day fast produced a significant rise in RBC ergothioneine of +1.0 mg. % (t = 4.2, n = 12). Six sham operations caused no significant change in RBC ergothioneine (t = <1.0). The average RBC ergothioneine in 15 mature nondiabetic rats was 5.8 ± 1.3 mg. %; a significantly lower level of 4.2 ± 1.0 mg. % was found in 8 depancreatized rats with nonfasting blood glucose concentrations higher than 280 mg. % (t = 3.1). The RBC ergothioneine in 12 rats decreased an average of 0.2 mg. %, 8–12 days following the injection of sufficient alloxan to raise the blood glucose to 350–710 mg. %. This was not considered to be a significant change (t = <1.0). (Supported by the Diabetic Research Foundation of Portland, Oregon).

Inhomogeneity of alveolar air: spot samples by catheter. A. A. BECHTEL (introduced by E. A. REED). *Dept. of Physiology, Hahnemann Med. College, Philadelphia, Pa.*

In dogs under Nembutal, two simultaneous samples from a single expiration drawn through catheters (without cuffs) from primary or secondary bronchi show differences in composition even when the catheter tips are tied together at the same depth. The catheters are polyethylene tubing, 1.57 mm. o.d. Samples of about 3-cc. volume are drawn simultaneously by means of a double-bore stopcock sealed to a pair of sampling bottles. Dead space (0.2–0.4 cc.) is washed with late expired air just before samples are taken. Analyses are done in duplicate on the Scholander ½-cc. analyzer standardized against atmospheric air. Differences between duplicates averaged less than .04% for both CO_2 and O_2 in experiments summarized here. Differences between simultaneous samples through catheters tied together at the same level in a primary or secondary bronchus (5 dogs) averaged 0.10% CO_2 in 33 pairs with greatest difference 0.37%; 0.165% O_2 in 28 pairs, greatest difference 0.63%. These values suggest actual differences in the composition of portions of the alveolar air. Pairs taken from catheters placed in a primary or secondary bronchus in each lung show differences (37 pairs, 7 dogs) averaging 0.17% CO_2 (maximum difference 0.74%) and 0.28% O_2 (maximum difference 1.00%). In a dog with a 46-month-old ligature on left pulmonary artery simultaneous samples from the 2 lungs gave differences averaging in 3 end-expiratory pairs, 1.49 ± .03% CO_2, 3.42 ± .24% O_2. On another day, with catheters in secondary bronchi, left and right lungs, the differences (4 pairs) averaged 0.89% CO_2 and 2.05% O_2. All differences were consistent with assumption of decreased gas transfer efficiency on ligated side.

Gas exchange during respiratory arrest with deep O_2 insufflation. A. A. BECHTEL* AND J. C. SCOTT. *Dept. of Physiology, Hahnemann Med. College, Philadelphia, Pa.*

Dogs have been maintained in respiratory arrest, without either normal or artificial respiratory movements, for as long as 7½ hours, by O_2 insufflation of 3 l/min. delivered from a catheter tip near the tracheal bifurcation. In the 7½-hour dog, gas samples of 3-cc. volume were drawn into evacuated bottles through fine catheters whose tips were 2½″ below the end of the O_2 supply tube in a primary (or a secondary) bronchus. Blood samples were taken from the femoral artery and vein. Gas analyses were done on the Scholander ½-cc. analyser, with duplicate differences averaging 0.024% for this series. Van Slyke manometric determinations show that arterial blood O_2 saturation was 98.1% at 5 and at 6 hours, falling to 86.9% at 7, and 83.7% at 7½ hours, when heart beat ceased to be palpable. (E.C.G., then showed ventricular ectopics.) Venous O_2 saturation, at 5, 6 and 7 hours was 36.6%, 31.4% and 22.5%. Venous CO_2 exceeded 105 vol. % at 7 hours. A-V CO_2 difference had disappeared at 6 hours and had been reversed at 7 hours (cf. DRAPER AND WHITEHEAD, *Anesthesiology* 10: 54, 1949). The CO_2 at the site of gas sampling was 4.39% after 5 minutes in respiratory arrest, 12.92% at 35 minutes, and 9.08% at 65 minutes. At 4, 5, 6, 7 and 7½ hours, it was 1.76, 1.43, 1.40, 2.17 and 1.95%. It is evident that a steep CO_2 gradient existed, from the site of sampling, to the site of blood-gas transfer at the alveolocapillary membranes.

Effect of applied external pressure on cardiovascular dynamics. JOHN R. BEEM, CARL R. HONIG* AND DEAN F. DAVIES. *Hypertension Div., Dept. of Internal Medicine, Washington Univ. School of Medicine, and St. Louis City Hospital, St. Louis, Mo.*

Effects of applied abdominal and peripheral pressure on cardiovascular hemodynamics and respiration were measured by a photokymograph in 11 subjects in the resting horizontal position. Ballistocardiographs were recorded by means of an electronic transducer, and blood pressures by a strain gauge with intra-arterial catheter. Photoelectric plethysmographs, electrocardiograms and pneumographs were recorded by the usual methods. Subjects were convalescent or preopera-

tive, afebrile men between the ages of 17 and 39 who had no cardiac or pulmonary diseases. Tracings were taken during inflation of specially constructed bladders about calves, thighs and abdomen, to 100 mm. Hg; recovery tracings after release of pressure were taken frequently. When both leg and abdominal bladders were inflated the effects on cardiac output, blood pressure and plethysmographs were greater than when either leg or abdominal bladders were inflated alone. Cardiac output generally increased immediately and systolic and diastolic blood pressures always increased when the abdominal bladder was inflated alone or in combination with the leg bladders. Inflation of leg bladders alone had negligible effect on cardiac output and slight transient effects on blood pressure. Whenever leg bladders were filled, toe plethysmographs indicated congestion. Compensation occurred rapidly in all parameters measured after the initial filling. On release of pressure the effects noted were in the opposite direction. Electrocardiographs frequently showed evidence of change in axis deviation when the abdominal bladder was inflated. Pulse wave contours usually showed a descent and deepening of the dichrotic notch when the abdominal bladder was inflated. This returned to its previous pattern on release of pressure.

Analysis of the kinetics and thermodynamics of the catalase-hydrogen peroxide system. ROLAND F. BEERS, JR.* AND IRWIN W. SIZER. *Dept. of Biology, Massachusetts Inst. of Technology, Cambridge.*

Based on the findings (CHANCE, B. *Acta Chem. Scandinav.* 1: 236, 1947; BEERS, R. F., JR. AND I. W. SIZER. *J. Biol. Chem.* In press) that *a*) the velocity constant, k_s, for the breakdown of hydrogen peroxide by catalase, E_t, is independent of substrate concentration and, that therefore *b*) the observed relative concentrations of the free enzyme, E_f, and the primary enzyme substrate complex, C_1, are maintained constant by a steady state process, equations may be derived which can determine the magnitude of the rate of formation, k_1, and breakdown, k_2, of the primary complex. *1.* $k_1 = k_s/2(E_f/E_t)$; $k_2 = k_s/2(C_1/E_t)$. Since it can be shown that: *2.* $k_s = 2k_1k_2/(k_1 + k_2)$, then the ratio of contributions of each rate process to the activation energy of k_s is k_2/k_1. However, *3.* $k_2/k_1 = E_f/C_1$. Therefore, a measure of E_f/C_1 as a function of temperature will establish whether the activation energies of k_1 and k_2 are the same. By means of the notatin system, which was used to supply a constant source of hydrogen peroxide (CHANCE, B. *Biochem. J.*, 46: 387, 1950), we have found in preliminary experiments no significant change in E_f/C_1 over the temperature range of 0°–50°, indicating that the activation energies of the 2 rate processes are approximately equal to that of the over-all process, k_s. The free energies and entropies of activation of the 2 rate processes differ by not more than 10%. (Work done under American Cancer Society Fellowship, recommended by the Committee on Growth of the National Research Council.)

An approach to O_2 consumption of the "active protoplasmic mass" (APM). ALBERT R. BEHNKE. *Naval Technical Unit, Heidelberg, Germany.*

Specific gravity and body water determinations provide an accurate measure of excess body fat and hence of the lean body mass (LBM), which in adults incorporates as a constant percentage the APM. Data obtained by Behnke and Welham, and Osserman *et al.* on two groups of Naval personnel (123 total; av. age 29, ht. 176.3 cm., wt. 75.5 kg. LBM 63 kg., % fat, 16.5—about 4% higher than average for general population of same age) show that predicted BMR divided by 1.20 (Cal/kg. LBM), '*K*', gives values for the LBM, 90% of which agree within ±10% of the densiometric LBM. This implies proportionality between SA and LBM made possible by the manner of distribution of relatively inert body fat in relation to LBM/ht. in the general population. LBM/ht. varies from 1.6 to 2.3 units as does SA. In the 10% divergent results, 'off average' individuals, measured BMR is required to determine whether or not SA or LBM better represents 'metabolic size'. Predicted '*K*' for a group varies from 1.10 to 1.23 depending upon the average fat content in relation to age. Age and sex differences in adults can be accounted for mainly on the basis of variation of excess fat content, i.e. O_2/kg. LBM is constant within limitations above outlined. Determination of standard (basal) metabolism makes possible an estimate of the LBM, its components, and those functions and entities which now in healthy adults are related to surface area, gross body weight or fractional powers of the body weight and which are subject to distortion by 'off' average body fat content in relation to LBM/ht.

Colloidal salts as plasma substitutes. VIVIAN G. BEHRMANN AND FRANK W. HARTMAN.* *Labs. of Henry Ford Hosp., Detroit, Mich.*

Since the macromolecular plasma extenders (dextran, polyvinyl pyrrolidone, pectin) resist metabolic degradation and show varying degrees of tissue storage, the search for a plasma substitute, at least partially utilized and less prone to storage, seemed worthwhile. A neutral colloidal salt of ammonium or an amino-acid base should be partially metabolized, thus sparing nitrogen and minimizing tissue retention. Acid hydrolysates (av. mol. wt., 50,000–60,000) of pectin and the mucilage plantago psyllium, prepared according to Pauling's hydrolyzation method, have been con-

verted with excess ammonium or amino-acid bases to neutral colloidal salts which are precipitated, washed, and desiccated with alcohol. These salts are stable, form clear solutions and are readily adjusted within physiologic limits of pH, viscosity, and osmotic pressure. Urinary excretion rates (dog) indicate that plantago is eliminated more rapidly than pectin although rabbits, injected tri-weekly with 3% plantago for 2–8 weeks, showed moderate storage up to 7–10 days but neither chronic retention nor enlargement of liver or other organs. Survival experiments less drastic than those of Govier were performed on thiobarbiturized dogs. Three hemorrhages, *1)* 2½–3% of body weight, *2)* 70% of the first and *3)* 48% of the first, each immediately replaced with a colloid salt in Ringer's solution, were carried out at 30 minute intervals. The criterion for survival was 12 hours after the third replacement. Preliminary results show that pectin derivatives are more effective than plantago. Further studies to determine the relative merits of these colloidal salts as compared with other proposed plasma extenders are in progress.

Blood flow and oxygen consumption of brain in relation to adrenal cortical action. JOHN R. BERGEN,* CHARLOTTE HUNT* AND HUDSON HOAGLAND. *Worcester Fndn. for Experimental Biology, Shrewsbury, Mass.*

Brain homogenates and slices taken from normal and adrenalectomized rats were found to show no differences in rates of oxygen consumption. *In vivo* studies, however, indicate a reduction in blood flow through the brain of 61% in adrenalectomized rats, and a decrease in brain oxygen consumption of 46%. Both differences are significant ($P < 0.005$). The rate of blood flow from the left posterior facial vein draining the brain sinuses was measured after ligating all other veins from the head. Arterial-venous differences in blood oxygen content were made by the Scholander method. While more oxygen is removed by the brain from the slowly flowing blood in the adrenalectomized rats, the total reduction in blood flow is sufficiently great substantially to reduce the brain's oxygen supply. The administration of 0.25 ml. Upjohn's lipoadrenal extract to adrenalectomized rats restores in 2 or 3 hours the blood flow to normal. Studies of effects of crystalline steroids are now under way. Elsewhere we have reported a slowing of the EEG in adrenalectomized rats and its restoration by adrenal cortical extract in amounts restoring normal blood flow. The degree of slowing of the EEG is consistent with the view that it results from anoxia mediated by the slowed cerebral circulation following adrenalectomy. (This work has been aided by a contract with the Office of Naval Research, and a cooperative project with the National Institute of Mental Health, U. S. Public Health Service.)

Pressure volume relationship and stress relaxation in pulmonary vascular bed of the dog. ERIK BERGLUND* AND STANLEY J. SARNOFF (introduced by JAN NYBOER). *Dept. of Physiology, Harvard School of Public Health, Boston, Mass.*

After ligating a large cannula in the main pulmonary artery and another in the left auricle, the mitral valve was occluded, and the cannulae were connected by nondistensible tubing to a nondistensible syringe-driven pump system. The latter was filled with blood previously obtained from the same dog. Ventilation of these isolated lungs *in situ* was maintained by intermittent positive pressure applied to the airway through a tracheal cannula. Pressures were then measured in the pulmonary artery and left auricle as 20 cc. increments of blood were injected into the system at 10-second intervals. This was followed by 20 cc. withdrawals at 10-second intervals. Significant differences were noted between the injection and withdrawal curves. These differences were most readily explained mainly by the phenomenon of stress relaxation of the pulmonary blood vessels and left auricle. Supportive evidence for the occurrence of this phenomenon will be presented as well as reasons for believing that the withdrawal type of pressure volume curve is of greater physiological significance. The yield point, or that pressure above which stress relaxation occurs, was found to be between 15 and 30 mm. Hg. A proportionality between the pressure level and the degree of stress relaxation that occurred was established. Varying the temperature of the perfusing blood from 28.9° to 39.4° C. did not significantly alter the curves obtained.

Effects of *l*-arterenol and *l*-epinephrine on the normal and denervated kidney. ROBERT M. BERNE. *Dept. of Physiology, Western Reserve Univ. Med. School, Cleveland, Ohio.*

Studies were done on anesthetized (pentobarbital or chloralose) and trained unanesthetized dogs with exteriorized bladders. The left kidney was denervated and the animal splenectomized 9–47 days prior to the experiment. The right kidney served as a control. Diuresis was accomplished in the anesthetized dogs by normal saline and mannitol intravenously, and in the unanesthetized dogs by hypertonic (1.5–2.0%) saline, water by mouth, or both. Creatinine and PAH were used to measure glomerular filtration rate (GFR) and renal plasma flow (RPF), respectively. Sodium was determined by a flame photometer using an internal standard. U.S.P. epinephrine, *l*-epinephrine, and *l*-arterenol were administered intravenously at rates of 0.2, 0.5, 1.0, and 2.0 γ/kg/min. *L*-arterenol in a dose of 0.5–1.0 γ/kg/min. raised the GFR on the normal side but decreased it on the denervated side in the anesthetized dog. Larger doses of *l*-arterenol and all doses of *l*-epinephrine used had either no effect or decreased the GFR bilaterally. The un-

anesthetized dog showed a similar response to *l*-arterenol but exhibited greater resistance to *l*-epinephrine RPF was depressed more on the denervated side with both *l*-arterenol and *l*-epinephrine, but the former produced a greater effect, especially in the unanesthetized dog. Urine flow and sodium excretion were usually decreased by *l*-arterenol and *l*-epinephrine, and in many instances in the presence of an elevated GFR. These responses were more marked on the denervated side. In some experiments where the GFR was considerably increased, urine flow and sodium excretion were augmented.

Elimination (metabolism and excretion) of *p*-aminobenzoic acid by the dog. KARL H. BEYER, ELIZABETH K. TILLSON,* HORACE F. RUSSO,* GRACE S. SCHUCHARDT* AND ALICE A. PITT.* *Pharmacology Section, Research Div., Sharp & Dohme, Inc., West Point, Pa.*

Preliminary experiments confirmed the apparent increase in '*p*-aminobenzoate (PAB)' clearance with time. This increase in PAB/creatinine clearance ratio (CR) has been ascribed to the formation and more rapid excretion of *p*-aminohippurate (PAH) which is not distinguished from PAB by simple analytical procedures. When neither benemid nor carinamide inhibited the increase in PAB CR we turned to the differential analytical method of Cohen and McGilvery to study the metabolism and excretion of PAB and, presumably, PAH. Thereby it was found that following the administration of PAB the actual PAB CR was less than 0.09, instead of 0.3 or greater, as previously reported. With increasing synthesis and plasma level the clearance of apparent PAH was depressed in a manner similar to that for PAH per se, but the magnitude of the clearances was less than anticipated. Chromatographic analysis of urine following administration of PAB revealed two peaks: one corresponding to PAB analytically; the other identified as its glucuronide instead of PAH. The Cohen and McGilvery method did not distinguish between the two possible metabolites. The proportion of PAB to its glucuronide in urine decreased with time but was not influenced notably by carinamide or benemid. It was concluded that the renal clearance of PAB by the dog was extremely low and that metabolism to its more rapidly cleared glucuronide accounted predominantly for its elimination when administered in large doses to dogs.

Effect of normal and modified bovine serum albumin on B-glucuronidase activity. A. L. BEYLER* AND C. M. SZEGO. *Dept. of Zoology, Univ. of California, Los Angeles.*

In experiments designed to test the effects of steroid hormones *in vitro* on the B-glucuronidase activity of certain accessory reproductive organs, the possibility of employing bovine serum albumin (Armour) to enhance the solubility of the steroid substances was examined. However, 0.1 % albumin itself when incubated with the crude enzyme and phenolphthalein glucuronide at pH 4.5, increased the rate of hydrolysis 39 %. Albumin (0.1%) completely restored the original glucuronidase activity of tissue homogenates which had been refrigerated for several weeks. In an attempt to characterize this albumin effect, the pH-activity curves for the enzyme with and without albumin were constructed. The comparison of the 2 curves revealed that the pH optimum for the glucuronidase was unchanged by albumin, but the shape of the curve was considerably modified. The albumin effect was most pronounced at pH 3.8 (+228 %) with a linear depression of the percentage difference occurring as the pH increased to 6.0 (+2 %). Since this finding suggested that the albumin effect might be related to the dissociation of basic groups, the effect of amino-blocked and carboxyl-blocked albumin (furnished by Dr. B. Levedahl) was measured. Blocking the amino groups of albumin by acetylation resulted in 78 % depression in the original albumin effect, whereas blockade of the carboxyl groups by methylation enhanced the albumin effect 51 %. These results are interpreted to indicate that the ability of bovine serum albumin to affect B-glucuronidase is in some way related to the net positive charge on the albumin molecule. (This investigation was aided in part by a grant from the Cancer Research Coordinating Committee, University of California.)

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Respiration of rat thymus in relation to body weight: effect of adrenalectomy. MARION K. BIRMINGHAM AND MARIE LOUISE DESBARATS (introduced by ORVILLE F. DENSTEDT). *Allan Memorial Inst., McGill Univ., Montreal, Canada.*

The rate of oxygen uptake of thymus suspensions in glucose-containing and glucose-free Krebs Ringer phosphate medium was determined in 47 male rats weighing between 25 and 450 grams. The respiration rate decreased as the body size increased and was less well maintained in the larger animals. The absence of glucose from the medium did not affect the respiration rate of thymus suspensions from rats under 50 grams, but became inhibitory as the animals increased in weight. There was also a significant positive correlation between respiration rate per unit weight of thymus and thymus weight in animals weighing over 200 grams, but not in rats weighing less, indicating that the respiratory activity of the thymus varied with its state of involution. Adrenalectomy, which caused an approximately twofold increase in thymus weight, was found to have no effect on the respiration rate in animals weighing between 50 and 225 grams. (This work was supported by funds from the National Research Council of Canada.)

Upper lethal temperatures of some British Columbia fresh-water fishes. EDGAR C. BLACK. *Dept. of Physiology, Univ. of British Columbia, and British Columbia Game Dept., Vancouver, B.C.*

Using Hathaway's method as modified by Fry and Brett, the upper lethal temperature was measured for 13 species of fish captured from southern Okanagan lakes during the summer months of 1951. Upper lethal temperature is here defined as the temperature at which 50% of a sample of 6–10 fish survived exposure to a given temperature for 24 hours. Fish were held at 11° C. till used. One temperature of acclimation was used for 9 species and 2 temperatures for 4 species. The approximate temperatures of acclimation (in parentheses) and the respective upper lethal temperatures expressed in degrees C. are: Kamloops trout fingerling, (11°) 24.0°; fine-scaled sucker, (14°) 26.9°; chub, (14°) 27.1°; silver-grey minnow, (14°) 28.3°; redside shiner, (14°) 27.6°; prickly sculpin, (18–19°) 24.1°; coarse-scaled sucker, (19°) 29.4°; largemouth bass, (20–21°) 28.9°; squawfish, (19–22°) 29.3°; northern black catfish, (23°) 35.0°; yellow perch (22–24°) 29.2°; pumpkinseed, (24°) 30.2°; carp, (26°) 35.7°. These data are compared with results obtained by other workers and with data for carbon dioxide asphyxiation curves determined for the same species under the same conditions. (This study was supported by a grant from the Natl. Research Council, Ottawa, Canada.)

Some effects of CCK 179 and pentobarbital anesthesia on renal function in dogs. WILLIAM D. BLAKE. *Lab. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.*

CCK 179 (kindly supplied by Sandoz Pharmaceuticals) is a mixture of dihydrogenated ergot alkaloids. Experiments were carried out on trained female dogs whether or not pentobarbital was given. In some only excretion rates of 'endogenous creatinine,' sodium, potassium and chloride were estimated on urine samples collected at 1, 2, 4 and 6 hours. In others urine collections were of short duration and the usual renal clearance techniques used to estimate filtration rate and plasma flow. To some dogs no water or salt was given (hydropenic), to others water (water loaded) or saline (saline loaded) was given by stomach tube. Anesthesia per se did not influence initial water or salt excretion in hydropenic animals but did bring about marked reduction in the excretion of these substances when either a water or saline load was imposed, i.e. anesthesia largely prevented the rapid elimination of administered water or NaCl. When CCK was given to anesthetized animals, excretion rates of water, sodium and chloride increased significantly under all conditions. CCK, thus, elevated water and salt loss to above unanesthetized control rates in hydropenic dogs but in water and saline loaded dogs only restored excretion rates close to unanesthetized control values. CCK appeared to have little effect on potassium excretion. In unanesthetized dogs the effect was variable. During anesthesia CCK may increase water and sodium output, in part, by blocking neural impulses to the kidney.

Comparison of effects of epinephrine and norepinephrine on carbohydrate metabolism in the rat. WALTER L. BLOOM* AND JANE A. RUSSELL. *Dept. of Biochemistry, Emory Univ. School of Medicine, Ga.*

l-Epinephrine or *l*-norepinephrine, 0.2 mg/kg., was given subcutaneously to unfasted rats. In one series, the glucose and lactic acid concentrations of the blood were determined initially and at 45, 90 and 180 minutes after treatment. After norepinephrine the maximum increase in blood glucose was less than one-third that after epinephrine, and no effect on blood lactic acid was demonstrated. Identical results were obtained in normal and in adrenodemedullated rats. In another series of normal rats, the gastrocnemius muscle, heart, diaphragm and liver were taken 4 hours after treatment for determination of total and of trichloroacetic acid extractable glycogen. After epinephrine the glycogen of the gastrocnemius muscle and diaphragm was diminished; but norepinephrine was without effect on these tissues. In the heart both epinephrine and norepinephrine induced significant increases in the glycogen content. No changes in liver glycogen were seen after either hormone. In fasted normal rats 4 hours after the induction of hypoglycemia by insulin, the gastrocnemius glycogen was diminished and that of the heart and diaphragm increased. In absence of the adrenal medullae, the glycogen of the gastrocnemius muscle and heart was unaffected by insulin, and that of the diaphragm increased to a greater extent than in normal animals. The alterations in glycogen content in all series of experiments were attributable almost entirely to changes in the trichloroacetic acid extractable fractions. It appears therefore that norepinephrine is not glycogenolytic in muscle, and that in absence of the adrenal medullae, sympathetic stimulation by hypoglycemia does not result in the release of glycogenolytic quantities of epinephrine.

Light scattering studies on myosin-adenosine triphosphate system. JACOB JOSEPH BLUM (introduced by T. BENZINGER). *Physical Biochemistry Div., Naval Med. Research Inst., Bethesda, Md.*

Light scattering and dephosphorylation experiments on the ATP-myosin (Weber-Edsall type, .003–.02%, in .6 M KCl, *p*H 7.0, 10^{-3} M Ca^{++}) system have been conducted, and analyzed by the Zimm

method. These show that when ATP is added to the protein solution, *1)* the protein mol. wt. is unchanged, indicating no 'depolymerization into myosin and actin,' and *2)* the characteristic dimension (L) is rapidly and significantly changed; with 4.0×10^{-4} M ATP, employing existing theory, this change seems to be an extension. After a certain amount of dephosphorylation a substantial return toward the original L occurs. The kinetics of this return are strictly related to the concentration of ATP still subject to ATPase action of the myosin. From separate experiments (no ATP) it appears that changes in *p*H (range 7–10) cause changes in L (constant mol. wt.) comparable in magnitude to those induced by ATP. They indicate that charge variation results in shape variation, i.e., the particles are flexible, and deformable by electrostatic forces. Preliminary experiments indicate that protein extracted for 24 hours instead of 5 has a somewhat greater L and 5-fold greater mol. wt. but in response to ATP is indistinguishable from the 5 hour product. The higher mol. wt. of 24 hour protein is widely considered to result from an enrichment of myosin with 'actin,' supposedly a non-ATPase. Our results suggest instead that the differences are caused by aggregation of the myosin. Parallel ultracentrifugal studies further characterize the myosin, and offer tentative support to the foregoing conclusions.

Induction of experimental heart failure with arteriovenous fistulae. J. R. R. Bobb, C. W. Lillehei* and M. B. Visscher. *Depts. of Physiology and Surgery, Univ. of Minnesota Med. School, Minneapolis.*

One of the difficulties in the study of heart failure has been the absence of a satisfactory method for producing it in experimental animals. It was suggested that large arteriovenous fistulae be produced in dogs which would then be given sodium chloride orally in order to induce congestive heart failure. This was done by making aortacaval, bilateral iliac, iliac-femoral, single iliac and bilateral femoral fistulae and then giving salt which in some animals exceeded 100 grams daily. Typical signs of congestive failure were seen, such as dyspnea, orthopnea, rapid weight gain, edema, ascites, venous pressure elevation, cardiac dilatation as well as other signs of failure. Some animals, especially the older ones and those with larger fistulae, went into failure without the administration of salt. A number of these developed acute failure and died quickly. At autopsy the lungs were found to have a liver-like consistency and the bronchi and trachea contained a pink or red frothy fluid. This picture was also seen in the animals which died after showing failure for a longer period. Physiologic studies showed that after the fistulae were produced the cardiac output (direct Fick), pulmonary artery pressure, venous pressure, blood volume and thiocyanate space were increased but after signs of failure appeared the latter factors increased while the first two decreased.

Succinic dehydrogenase in mitotically active and blocked embryonic cells. J. H. Bodine. *Zoological Lab., State Univ. of Iowa, Iowa City.*

A study has been made of the succinic dehydrogenase activity of the developing embryo of the grasshopper, *Melanoplus differentialis*. Enzyme activity of mitotically active as well as blocked embryonic cells has been measured. Marked differences in enzyme activity are noted between active and blocked cells. Greatest activity seems present during the blocked or diapause state. Enzyme is located largely in the mitochondria while none has been demonstrated in nuclei. The action of various chemical reagents upon the succinoxidase activity has been studied.

Calcium balance in the aged male. Morton D. Bogdonoff,* Mary P. Nichols* and Nathan W. Shock. *Natl. Heart Inst., Natl. Insts. of Health, Bethesda, and Sec. on Gerontology, Baltimore City Hosp., Baltimore, Md.*

Metabolic balance studies were carried out over a 4-month period in 3 males aged 66, 73, and 81 years. The subjects were all ambulatory and after detailed clinical and laboratory tests were judged to be free of renal, gastro-intestinal and bone disorders that might have affected calcium metabolism except for x-ray evidence of minimal osteoporosis. Balance periods of 7 days each were run at calcium intakes of 107 mg/day for 5 periods, 849 mg/day for 4 periods, 1543 mg/day for 4 periods and 2494 mg/day for 2 periods. The calcium was provided in natural foods at all intake levels except the 2494 mg/day. All diets, urines and stools were analyzed for Ca, P and N. The caloric intake for all periods was 2600 cals/day and the nitrogen and phosphorous contents of the diets ranged from 13.6–16.5 gm/day and from 927–1931 mg/day respectively. All subjects were in positive N and P balance in all periods. All subjects were in negative Ca balance at an intake of 107 mg/day (av. 107 mg/day) even though the N and P balances were positive. With increasing levels of Ca intake the average Ca balances were +84, +160 and +354 mg/day respectively although the storage of N and P remained essentially the same. No significant change in serum Ca level (total) or daily urinary excretion of Ca were found with increased levels of Ca intake.

Analysis of the electrolyte changes in rats during cold stress. Walter M. Booker, Raymond L. Hayes*, Frances Dent Da Costa*, William

JONES*, RICHARD HILL* AND PATRICK TITUS*. *Depts. of Pharmacology and Oral Medicine, Colleges of Medicine and Dentistry, Howard Univ., Washington, D. C.*

Preliminary experiments reported from these laboratories have shown that in both intact and adrenalectomized mice potassium rises in blood, in heart and in skeletal muscle during cold stress. During recovery periods (1–2 hours) the potassium decreases almost to comparable levels with unstressed animals in the intact group, but fails to decrease in the same period of recovery in the adrenalectomized animals. Adrenalectomized animals, treated with cortisone show increases of potassium during stress periods, but there is some tendency to return toward normal (intact) levels during recovery periods. The changes in sodium are not outstanding in either intact or adrenalectomized animals except when cortisone is given; then there is increase in sodium in blood of intact animals. In the present series of experiments, using rats, attempt is being made to determine whether the increase in potassium in blood, heart and skeletal muscle during stress periods represents release from some other source in the body or increase in renal threshold to potassium. There is growing evidence that urinary potassium excretion decreases during stress and increases during recovery periods in intact animals. Cortisone apparently lowers the renal threshold to potassium during stress periods in both intact and adrenalectomized animals. In animals so treated there is less significant increase in blood and tissue potassium during stress periods. Experiments are underway to determine the significance of these observations.

Effect of digoxin upon rate of shortening of myosin B threads. WILLIAM J. BOWEN. *Natl. Insts. of Health, U. S. Public Health Service, Bethesda, Md.*

ATP causes threads of myosin B to shorten and the rate is affected by several metallic cations. Now it has been found that the cardiac glycoside, digoxin, causes an increase in the rate of shortening of myosin B threads made from skeletal muscle. This finding is based upon the study of 143 pairs of threads. The experimental thread of each pair was made to shorten by ATP in the presence of one μg. of digoxin/ml. The other shortened in the absence of digoxin. The order of study of experimental and control threads was alternated with each pair. The mean amount of shortening of threads 30, 60, 90 and 120 seconds after the application of ATP was greater ($P < 0.05$) in the presence than in the absence of digoxin. It has been shown (BOWEN, *Am. J. Physiol.* 165: 10, 1951) that the rate of shortening of myosin B threads proceeds at the rate of a first order chemical reaction. The time required for half reaction is a measure of the velocity of a first order reaction. In the presence of digoxin the mean time required for half shortening was 45 seconds, while that in the absence of digoxin was 54 seconds. Since no carbohydrate metabolism is known to occur in myosin B threads, digoxin must produce its effect by altering the contractile proteins or the union between ATP and myosin B.

Effects of controlled low oxygen breathing upon exchange of respiratory gases in humans. BEN VAUGHAN BRANSCOMB AND GEORGE W. WRIGHT (introduced by J. N. STANNARD). *Dept. of Physiology, E. L. Trudeau Fndn., Saranac Lake, N. Y.*

Minute ventilation was maintained at a selected constant rate and tidal volume throughout each experiment. Each subject fasted 12 or more hours and was recumbent 60 minutes prior to the measurements. Gas exchange was measured continuously by the open circuit method throughout the following consecutive periods: *period A*, respiring room air without controlled breathing pattern for 20 minutes; *period B*, respiring room air (pO_2 = 148–153 mm. Hg) with controlled breathing pattern for 30 minutes; *period C*, respiring nitrogen-oxygen mixture (pO_2 = 105–115 mm. Hg) with controlled breathing pattern for 60 minutes; *period D*, respiring room air with or without controlled breathing pattern for 20 minutes. Arterial hemoglobin saturation was followed by oximetry and periodic direct measurement in several experiments. 15 studies on 6 normal male subjects were made. Carbon dioxide output remained remarkably constant throughout all periods. In the majority of the experiments there was a significant decrease of oxygen uptake during *period C*. This finding was not uniform as to quantitative aspects or repeatability. In 7 experiments the reduction of O_2 uptake was more than 10% of the control level, the greatest reduction being 40%. (Supported by contract N7onr-307, Task Order 3 NR 112-214 with Office of Naval Research.)

Effect of respiration on venous return to the heart. GERHARD A. BRECHER AND GEORGE MIXTER, JR.* *Dept. of Physiology, Western Reserve Univ. Med. School, Cleveland, Ohio.*

According to the classical aspiration theory, venous return increases with inspiration. The collapse theory of Holt as applied by Duomarco denies this possibility. The mechanism of venous return was studied in open and closed chest dogs. In open chest animals, changes in intrathoracic pressure were substituted for by direct aspiration at the peripheral end of the divided vena cava superior. Two distinctly different stages were observed when suction was applied to the peripheral end of the vessel. During the first, or 'depleting,' stage (characterized by a continuous reduction of

the vein's filling state) outflow from the vein increased in direct proportion to the pressure gradient, while resistance remained almost constant. The second, or 'collapsed,' stage was only entered after the veins had emptied part of their content and assumed a reduced filling state during which outflow remained constant and resistance increased with greater pressure gradients. In closed chest animals, flow through the superior vena cava was measured with a differential flowmeter. With normal inspirations, flow always increased progressively during the entire duration of inspiration due to depleting of the extrathoracic veins into the thoracic veins. Only toward the end of the inspiratory phase of forced, prolonged inspirations could the collapsed stage of the extrathoracic veins be reached, thus preventing a further flow increase.

Electrocardiograms which represent projection of the vectorcardiogram on any axis of the Einthoven triangle. DANIEL A. BRODY,* ROBERT C. LITTLE AND HALL S. TACKET.* *Depts. of Medicine and Physiology, Univ. of Tennessee, Memphis.*

The 'axostat,' a new instrument developed in this laboratory, produces any desired number of interpolated leads between the standard bipolar extremity leads. Both arms and left leg are connected by means of low resistance electrodes to corresponding terminals of a balanced 3-phase resistance network consisting of a Y within an inverted Δ. A sliding contact is adjustable to any position on the periphery of the network. Potential differences between this contact and the center of the Y, after a predetermined amount of compensating amplification, are coupled to an electronic electrocardiograph. Tracings obtained in this manner from electrical models and humans have been demonstrated to represent the projection of successive cardiac vectors upon a line passing from the center of the Y through the peripheral point. As anticipated, tracings made at 0°, 60° and 120° are virtually indistinguishable from, respectively, *leads I, II* and *III*. Transition point electrocardiograms (derived leads in which the area under a particular wave or complex of waves is algebraically zero) have proved especially valuable: They appear to be exceptionally sensitive to most factors which affect the wave under consideration. They also indicate rather precisely the direction of the mean vector of the wave since the axis of the lead is at right angles to the mean vector. Such information regarding orientation greatly reduces the amount of planimetry required in accurate calculation of ventricular gradients. (Supported in part by a grant from the Memphis Heart Association.)

Temperature changes in humans during exposure to a lowered partial pressure of oxygen in a cool environment. ARNOLD L. BROWN, JR.,* GORDON F. VAWTER,* AND JOHN P. MARBARGER. *Aeromedical and Physical Environment Lab., Univ. of Illinois, Chicago.*

Combined influence of reduced oxygen tension in inspired air with simultaneous exposure to cool environments has been demonstrated on small animals. In such experiments the rate of decrease in body temperature is greater than that which occurs during exposure to the same temperature in the presence of normal oxygen supply. Similar results have been reported in dogs and on a limited number of humans. A study of the effect of hypoxia on temperature regulation in normal humans during exposure to a cool environment was accomplished by subjecting two groups, numbering 10 unclothed subjects each, to an environmental temperature of 50°F. for 90 minutes. *Group I* subjects breathed room air so that the arterial oxygen saturation was normal, while that of *group II* was reduced to between 68 and 85% as measured by an oximeter. This was accomplished by reducing the barometric pressure in the cold room. At the end of 90 minutes, subjects in *group II* breathed 100% oxygen. Measurements were made of skin and rectal temperature, heart rate and blood pressure. Significant differences between *groups I* and *II* could not be demonstrated. Rectal temperature in both groups decreased 2° F. and skin temperature 12° F. Rectal temperature in some of the subjects in *group II* increased 0.2° to 0.3° F. following administration of 100% oxygen. This was attended by violent shivering. No outstanding changes occurred in blood pressure or heart rate. Human studies reported herein are at variance with similar extensive studies on animals.

Venous pressure during exercise in man: relation to blood volume, cardiac competence and pulmonary pressure. E. BROWN, J. HOPPER, JR. AND J. D. LANGE (introduced by J. J. SAMPSON). *Div. of Medicine, Univ. of California School of Medicine, San Francisco.*

In 17 experiments on healthy subjects, 10 on patients with diseases involving the left ventricle primarily (*group A*) and 19 on patients with either pulmonary disease or mitral stenosis (*group B*), the following were carried out: After subjects rested 30 minutes, blood volume was measured, using T-1824 and carbon monoxide. Venous pressure (V.P.), pulse rate, respiratory rate and depth, and chest position were recorded continuously during *a*) 5 min. of rest, *b*) 5 min. in which approximately 3500 foot-pounds of external work was performed (weight lifting in foot treadle), and *c*) 5 min. recovery. Resting V.P. exceeded 150 mm. saline in only 3 experiments involving hyper-

volemic cardiac patients. In healthy subjects, V.P. increased during exercise (maximum 75 mm. saline), remained elevated or fell considerably during exercise, but always fell to resting level or below within 60 sec. after stopping exercise. In *group A* patients, V.P. rose abnormally only when blood volume exceeded 20% of predicted normal; in *group B*, no correlation was found between blood volume and response of V.P. to exercise. In 2 patients, the height of the V.P. rise during exercise was reduced following reversion from auricular fibrillation to sinus rhythm. The presence and severity of pulmonary hypertension was reflected by the V.P. response to exercise in 5 of 6 patients whose mean pulmonary arterial pressures, measured directly, were between 17.5 and 58.5 mm. Hg. The results support the concept that whereas in cardiac patients resting V.P. may relate to blood volume, changes resulting from exercise are more dependent on the competence and work load of the right ventricle.

Carbon dioxide tolerance of the dog heart. E. B. Brown, Jr. and Fletcher A. Miller.* *Depts. of Physiology and Surgery, Univ. of Minnesota, Minneapolis.*

Electrocardiograms, arterial blood pH determinations and blood pressure recordings were obtained on dogs before and at intervals during a gradual increase in the carbon dioxide concentration of the inhaled gas mixture. The increase in CO_2 concentration was continued until cardiac arrest occurred. When severe respiratory depression became evident, the CO_2 in oxygen mixture was supplied by artificial respiration. This usually occurred when the CO_2 concentration exceeded 60%. When the CO_2 concentration was increased at a rate of approximately 1% per minute the average concentration at cardiac arrest was 85%. Arterial blood pH decreased from the average control value of 7.39 to 6.43 on the last sample drawn before cardiac arrest. Heart rate decreased from an average of 172 on air to 136 on 50% CO_2 to 73 at the time the last electrocardiogram was made, which in some instances included the last heart beat. A slight but regular increase in P–R interval appeared as the CO_2 concentration reached very high values. Little or no change in QRS interval was noted. No arrhythmias appeared on the high CO_2. Blood pressure remained high, usually above control levels, until a CO_2 concentration of approximately 70% was reached when it started to decline and fell precipitously as the CO_2 concentration exceeded this level. Three dogs breathed CO_2 in excess of 90% for periods of 4 to 9 minutes before cardiac arrest appeared.

Application of infra-red CO_2 analysis to lung compartment estimation. E. S. Brown (introduced by H. L. White). *Div. of Anesthesiology, Washington Univ. School of Medicine, St. Louis, Mo.*

A continuously recording CO_2 analyzer has been utilized in conjunction with simultaneous pneumotachograph recording to measure functional lung compartments in normal and abnormal subjects. Methods of estimating physiological dead space, 'kinetic dead space,' and alveolar space from these data are discussed. Changes in the calculated functional residual lung volume from the continuous CO_2 records have been correlated with those observed by flow meter and spirometer. Physiological dead space increases with increasing tidal volume and with increasing functional residual volume.

Purification and properties of lipemia clearing factor. Ray K. Brown, Edwin Boyle, Jr. and Christian B. Anfinsen, Jr. (introduced by James A. Shannon). *Sec. on Cellular Physiology, Natl. Heart Inst., Natl. Insts. of Health, Bethesda, Md.*

Heparin and the synthetic anticoagulants Treburon and Paritol upon injection produce a rapid clearing of the turbidity of lipemic plasma. Plasmas, withdrawn following heparin injection in normal cats, dogs, rats and humans, cause the *in vitro* clearing of lipemic plasmas at a rate proportional to the amount of heparin administered. No species specificity was observed in these carnivores. NIH strain rabbits produce little or no clearing factor. The turbidity and ultracentrifugal appearance of hypercholesterolemic rabbit plasma lipoproteins are only minimally altered by active preparations, whereas dog plasma lipoprotins are markedly altered. Heparinized blood perfused through the isolated hind limbs of rats contained no clearing factor. The organs producing clearing factor have been investigated both by perfusion and by *in vitro* incubation techniques. This clearing factor (dog plasma) has been partially purified by low temperature ethanol fractionation and falls in Fraction III—1, 2, 3 using Cohn's methods 6 and 9, or 10. The activity is destroyed by heating 3 minutes at 58° C., by chymotrypsin digestion, and by sodium chloride concentrations greater than 0.5 M. The activity withstands dialysis and lyophilization. Activity was determined by the rate of clearing of dog alimentary lipemia serum. The purified material does not clear ultracentrifugally floated lipoproteins of density less than 1.006 unless a substance present in the infranatant is added.

Age changes of total body fat in normal adult men. Josef Brozek and Ancel Keys. *Lab. of Physiological Hygiene, Univ. of Minnesota, Minneapolis.*

It has been shown (*Science* 112: 788, 1950) that middle-aged business and professional men of standard weight for age and height differ in their body composition from college students in their early twenties. There was a marked increase in the fat content of the body, estimated from the specific gravity. This trend has been studied in

age groups 18–22 (N = 41), 23–27 (N = 24), 43–47 (N = 21), 48–52 (N = 56), and 53-57 (N = 21). Each sample consisted of individuals with a body weight within ±10% of the standard weight. In all samples the average weight coincided very closely with the standard. The average specific gravity (obtained by using 1.5 l. as the value of residual air) decreased throughout the age range studied with the values of 1.082, 1.078, 1.048, 1.045, and 1.044, respectively. The trend is somewhat exaggerated because the value of the residual air also increases with age. Additional data will be presented with actual values of residual air determined at the time of underwater weighing. The increased fatness was reflected in thicker skinfolds and in a decreased difference between the circumference of the chest and the abdomen. The trend was evident also in data on submarine personnel, made available by Dr. Sarkis S. Sarkisian. For age groups 18–22 (N = 82), 23–27 (N = 72), 28–32 (N = 20) and 33–37 (N = 5) the specific gravity values were 1.063, 1.058, 1.058 and 1.044. (Supported by a grant from the U. S. Public Health Service.)

Malonate inhibition of egg cleavage and its reversal by succinate, fumarate and ATP. MANFRED BRUST AND ROBERT C. BARNETT (introduced by C. L. PROSSER). *Marine Biological Lab., Woods Hole, Mass.; Committee on Physico-Chemical Biology, Univ. of Illinois, Urbana; and Dept. of Physiology, Univ. of Chicago, Chicago, Ill.*

It has been shown that the division of *Arbacia* eggs is inhibited by low oxygen tension. This inhibition is completely reversed by added adenosine triphosphate (ATP) (BARNETT, *Federation Proc.* 10: 9, 1951). ATP is believed to be mainly produced through the Krebs tricarboxylic acid cycle in aerobic organisms. It was therefore considered of interest to test the functional significance of the Krebs cycle in egg division by means of malonate inhibition, and succinate and fumarate counteraction to malonate. Two extreme cases of respiratory response to fertilization were investigated: *Arbacia*, an echinoderm, which shows an increase in oxygen consumption, and *Chaetopterus*, an annelid, which shows a decrease. ATP, inorganic pyrophosphate, adenylic acid, and the sodium salts of malonic, succinic and fumaric acids were added to 0.1–0.2 per cent (by volume) suspensions of washed *Arbacia* and *Chaetopterus* eggs. Division of *Arbacia* and *Chaetopterus* eggs was completely inhibited by 0.1 M malonate in sea water adjusted for isotonicity. Malonate, at the same concentration, when added directly to sea water (hypertonic) was 50% less inhibitory. In both species of eggs isotonic succinate and fumarate, 2.7×10^{-2} M, completely reversed the inhibition of division caused by malonate. ATP, $1.8 - 5 \times 10^{-5}$ M, incompletely reversed malonate inhibition, whereas inorganic pyrophosphate and adenylic acid showed no counteraction in both species. These results may be tentatively explained by postulating at least two systems necessary for division, namely: an ATP generating system, or systems dependent upon ATP; and a synthetic system dependent on the Krebs cycle for the production of one or more substances necessary for division.

Studies on optical properties of active nerve. S. H. BRYANT* AND JULIAN M. TOBIAS. *Dept. of Physiology, Univ. of Chicago, Chicago, Ill.*

Following the detection of changes in optical properties of polarized nerve we have begun to study optical changes resulting from impulse conduction. Using white light impingent upon the surface of whole frog, spider crab and *carcinus* nerves, changes have been measured in the amount of light deviated (scattered, reflected, etc.) by the nerve at a right angle to the incident beam. It is hoped that ultimately such experiments will yield information concerning changes in ultrastructure accompanying activity. Results to date confirm the findings of Hill (*J. Physiol.* 1950) that the amount of light scattered by crab nerve changes as a function of activity. In addition, a similar phenomenon has been found using whole frog nerve (sheath intact). It is also found that the amount of physical stress (stretch, etc.) applied to the nerve influences the direction and quantity of the change. In a lax nerve (*Carcinus*) activity is accompanied by a dominant decrease in scattering. In a stretched nerve activity is accompanied by a dominant increase. Changes have been convincingly and repeatedly seen with a single stimulus. The interpretation of these findings will be discussed.

Bone carbonate turnover. DONALD L. BUCHANAN AND AKIRA NAKAO (introduced by HENRY T. RICKETTS). *Div. of Biological and Med. Research, Argonne Nat. Lab., Chicago, Ill.*

Adult rats and mice and infant mice were exposed in chambers for periods up to 6 months to air which contained $C^{14}O_2$. Control of the exposure conditions maintained a steady specific activity (s.a.) in the CO_2-bicarbonate system of these animals. In mice born in the labeled atmosphere, and raised there for periods of 1–6 months, the bone carbonate s.a. was constant at approximately 70% of the computed steady state s.a. of alveolar CO_2. Assuming this constant s.a. in growing bone to represent isotopic saturation, the carbonate of adult mouse and rat bones became 30% saturated in a week but only 40–45% saturated in 3 months. The uptake curves did not differ significantly between rats and mice nor between different bones in the rat. Exposures of partially grown animals showed that growth greatly stimulates the turnover of pre-existing bone carbonate presumably

because of structural rearrangement accompanying the growth process. In experiments where mice were exposed for various times and then analyzed at intervals following the exposure it was found that the long-term retention of isotopic carbonate in the entire skeleton was approximately proportional to the duration of the exposure regardless of the age of the animals when exposed. It may be concluded that the carbonate in new bone arises wholly or largely by incorporation of soluble inorganic carbon into precipitating bone salt, but that once laid down, a large fraction of such carbonate exchanges very slowly with the CO_2-bicarbonate system.

An analysis in depth of auditory and optic potential fields of cerebral cortex. NATHANIEL A. BUCHWALD* AND BERRY CAMPBELL. *Dept. of Anatomy, Univ. of Minneosta Med. School, Minneapolis.*

The spatial and temporal distribution of the potential fields of the layered structure of the cerebral cortex may be studied by deriving isopotential contour maps from families of potentials recorded at various depths by probing electrodes. The potentials are evoked by single stimuli and recorded as oscillographic traces. The maps allow analysis of the variation of positive and negative potential loci with depth and time beneath a single point on the cerebral surface. Series of maps made beneath different points on the surface show areal patterns. The technique for the study of the restricted geometry of the cerebral cortex has been derived from the method of contour mapping of potential fields within the spinal cord (CAMPBELL, *Anat. Rec.* 91: 77–88). Contour maps of the potential fields evoked by stimulation of the optic nerve in cats and recorded from several locations in the visual area as well as similar maps illustrating the potential field evoked by auditory stimulus (clicks) in the center and edge of the auditory area will be presented and discussed. Histological location of the needle electrode tracks permits correlation of potentials with cortical structure.

Pharmacologic studies on the canine tremor syndrome (motion picture). CARL A. BUNDE, BENEDICT E. ABREU,* GEORGE R. BURCH* AND ALICE B. RICHARDS.* *Research Dept., Pitman-Moore Co., Indianapolis, Ind.*

Examples are shown of a canine tremor syndrome resembling Parkinson's disease in man. Several cases are demonstrated before and after treatment with the 4-*n*-butoxy-β-(1-piperidyl) propiophenone hydrochloride, one of the two most active compounds in the series of propiophenones investigated (see ABREU *et al.*, nervous system effects of 4-alkoxy-β-(1-piperidyl) propiophenones). The 4-*n*-butoxy derivative, in doses of 0.5 mg/kg., i.v., is effective in diminishing the tremor of most and at doses ranging from 1.0–2.0 mg/kg., i.v., will abolish it in the majority of such dogs. Toxic reactions which appear when the dosage is increased include head tremor, nausea and vomiting and with even greater dosage clonic then tetanic convulsions.

Response of certain microorganisms to a pterine compound isolated from developing egg of grasshopper. L. E. BURGESS,* E. H. MCNEIL* AND D. T. ROLFE. *Dept. of Physiology, Meharry Med. College, Nashville, Tenn.*

A crystalline pterine compound has been isolated from the developing egg of the grasshopper (BURGESS, *Arch. Biochem.* 20: 347, 1949) and has been shown to be active in initial tests on the stimulation of growth in *streptococcus faecalis*, *Escherichia coli* and *Lactobacillus Delbruckii*. This compound appears late in the developing egg of the grasshopper and shows a marked increase in concentration during the same period in which the riboflavin content is decreasing (BODINE *et al. Physiol. Zool.* 20: 1947). The present investigation was undertaken in an attempt to determine whether this pterine compound has a riboflavin-like activity for the stimulation of growth in certain bacteria. The basal medium used was similar to that of Burkholder *et al.* (*Arch. Biochem.* 7: 287), and supplemented with the pterine compound in increasing concentrations. The inoculum was prepared from an 18 hour culture of the micro-organisms grown in enriched nutrient broth. One-tenth ml. of suspension of the micro-organisms in sterile physiological saline was pipetted aseptically into 10 ml. of media in each assay tube. Turbidimetric readings were taken at 18, 24, 48 and 72 hours using Klett-Summerson photoelectric colorimeter. Titrimetric determination was made at the end of 72 hours using 0.1N NaOH. The evidence obtained tends to show that this compound has the ability to stimulate growth and acid production of these microorganisms. (Aided by a grant from the National Institutes of Health, U. S. Public Health Service.)

Action of DDT on the blood of the chicken. H. BURLINGTON AND V. F. LINDEMAN. *Dept. of Zoology, Syracuse Univ., Syracuse, N. Y.*

The possibility of detecting accumulated DDT by characteristic alterations of blood constituents was tested by studying the effects of chronic poisoning on the blood of Brown Leghorn and New Hampshire Red Cockerels. Subcutaneous injections of purified (M.P. 109.5° C.) DDT were initiated on the 8th day posthatch and continued until analyses were performed at 62–89 days of age, when a total of 2–3 gm. had been administered. Body weight was unaffected by treatment, while testes development, as seen in earlier experiments, was markedly retarded. No significant alterations of red cell number, hemoglobin levels, blood sugar, plasma proteins, plasma calcium, phosphorous,

fibrinogen, or prothrombin time resulted from treatment. Thus, accumulation of the poison seems not to be reflected by characteristic deviations of the components studied. Furthermore, the possibility of the insecticide exerting an estrogenic action (BURLINGTON AND LINDEMAN. *Proc. Soc. Exper. Biol. & Med.* 74: 48, 1950) is contraindicated by the absence of effect on several of the blood constituents known to be modified by estrogen administration. The fact that circulating calcium levels remained undisturbed even in birds manifesting acute symptoms suggests further that contrary to what has been proposed for the rat, a hypocalcemia is not involved in producing the tremors associated with DDT intoxication in the chicken.

Effect of hypoxia and hypercapnea on standing potential of man. HAROLD S. BURR* AND ROBERT B. LIVINGSTON. *Section of Neuroanatomy and Lab. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.*

Experiments were designed to explore the possibility of using biological potentials as a means to activate a signal warning of anoxic anoxia. An attempt was made to determine whether there might be a significant change in the relatively steady standing potential in response to the inhalation of gas mixtures low in oxygen under conditions roughly approximating those of flight. Experiments were conducted at simulated altitude and at sea level. In many subjects altitude per se appeared responsible for a significant and progressively increasing change in potential beginning at about 15,000 feet. This change occurred even though the inspired pO_2 was kept relatively constant, and did not occur in naive subjects when decompression was merely simulated but not carried out. A factor which involved only 3 out of 18 medical student subjects occasionally produced modification of their potential, presumably as a result of emotional stress. Withdrawal of oxygen at 26,000 feet caused a shift in potential which in most subjects was of a significant degree before the subject was incapacitated by lack of oxygen. Such shifts in potential appear to be a consequence of a markedly reduced pO_2 and not merely a change in pO_2. Alterations in standing potential of a similar order of magnitude but [illegible] more rapid in onset, occurred in most subjects as a result of the inspiration of gases containing increased quantities of CO_2. The use of this technique as an anoxia warning device will depend upon further instrumentation.

General theory of the shape of pressure-flow curves in vascular beds. ALAN C. BURTON, F. GIRLING* AND W. JERRARD.* *Dept. of Biophysics, Med. School, Univ. of Western Ontario, London, Canada.*

Our work of recent years has yielded a wide variety of curves of pressure and flow for the leg of frog and rabbit, for the rabbit ear and for the human arm, under different degrees of vasomotor tone produced by sympathetic stimulation or by pressor drugs. A generalization is possible as to the sequence of changes in shape under increasing tone. One feature, the increasing intercept on the pressure axis, has been explained by the fundamentals of the physical equilibrium of the small vessels (critical closing pressure). We now find that the variety and sequence of shapes can be explained starting from the same basis and using Poiseuille's law. A 'single equivalent vessel' is imagined which would offer the same resistance to flow as the whole bed, and endowed with an 'elastic diagram' of the kind known to apply to blood vessels. This would give a series of pressure-flow curves under increasing tone corresponding to those found. Fitting such a crude theory to the data gives information as to the elastic properties of the 'equivalent single vessel.' An exception to the generalization is the case of a vascular bed containing two elements in parallel with widely different critical closing pressures, as in the mesentery. The pressure-flow curve of the mesentery of the frog, when tone is present, shows an abrupt discontinuity indicating the critical closure of the A–V shunts separately from the capillary bed. (Supported by the Life Insurance Medical Research Fund.)

Protective mechanisms against radiation in biological systems. BERRY CAMPBELL. *Dept. of Anatomy, Univ. of Minnesota Med. School, Minneapolis.*

Consideration of the survival problem of biological systems which have operated in a minimum radiation flux of the order of magnitude of 10^{-4} r/day or 10^{7} r during geologic time leads to appreciation of the efficiency of the protective mechanisms. The dangers fall into two main classes, the tendency for recessive lethals to accumulate and the chemical poisoning effects of ionization. The latter is not a problem in these considerations for the low dosage rate does not result in cumulative effects. The mutagenic effect, the main product of which consists of recessive lethals, is a real danger and sums with thermal agitation and other factors producing genic degradation. The main protective mechanism is the alternation of haploid and diploid generations. Because of the complex chemical and morphogenetic sequences in the male gamete and the unmasking of recessive genes, natural selection has an extremely strong effect at this stage. The high incidence of morphological abnormalities of the sperm is interpreted as a sign of high mutation rate. The calculated effect of low versus high gene number as well as the value of large chromosome numbers will be discussed. The survival value of alternating

haploidy and diploidy over a steady state in either stage will be shown. Sexual reproduction, which is universal among complex animals and in most plants, is thus to be looked upon as a protective mechanism toward this environmental factor.

Studies on in vitro inactivation of alpha-estradiol. A. CANTAROW, K. E. PASCHKIS, T. L. WILLIAMS* AND W. P. HAVENS, JR.* *Jefferson Med. College, Philadelphia, Pa.*

In previous studies, alpha-estradiol has been incubated with liver slices, homogenates or brei for periods of 1–3 hours. Under such circumstances, 65–100% of the original biological activity may be lost, the chief metabolite identified thus far being estrone. In the present study, alpha-estradiol was incubated for ¼-, ½-, and 2-hour periods with slices and homogenates of normal and acetaminofluorene-treated rats. The residual estrogens were determined by bioassay and fluorometric procedures. It was found that loss of biological activity occurs rapidly, falling to an average of 14% of the original activity in 15 minutes, to 12% at 30 minutes, and to 4% at 2 hours. The corresponding values obtained by the fluorometric procedure were 56, 39, and 12% respectively. Inasmuch as estradiol, estrone and estriol, which differ considerably in biological activity, yield much the same fluorescence values, the observed discrepancies between the results obtained by these two procedures may be due to conversion of estradiol to one or other of the less potent estrogens. This point is under investigation. There was no significant difference between the results obtained with the livers of normal and those of acetaminofluorene-treated rats.

Factors influencing excretion of chloride. WALTER H. CARGILL (introduced by G. CLINTON KNOWLTON). *Lawson V. A. Hospital, Chamblee, Ga.*

In an attempt to elucidate the renal regulation of electrolyte and water balance rates of glomerular filtration (inulin and endogenous creatinine clearances), urine flow and chloride excretion have been determined before and during the following procedures: ingestion of water and isotonic saline solution, standing in air and immersed in water, abdominal compression, the application of venous tourniquets to the lower extremities, and the acute and chronic administration of desoxycorticosterone and cortisone. Although no exact correlation could be demonstrated, alteration of the filtration rate is usually accompanied by parallel changes in urine flow and chloride excretion. Under conditions in which the filtration rate is decreased the renal response to a salt load produced by the intravenous infusion of hypertonic saline solution is diminished or abolished. Attempts to demonstrate a relationship between these functions and the extracellular fluid volume (inulin space) were unsuccessful.

Adaptive mechanisms in cold environments. LOREN D. CARLSON, HENRY L. BURNS*, ALLEN C. YOUNG* AND THOMAS H. HOLMES.* *Dept. of Physiology and Biophysics, Univ. of Washington School of Medicine, and Dept. of Psychiatry, Univ. of Washington School of Medicine, Seattle.*

An hypothesis concerning adaptation to cold in the human has been formulated utilizing data from the literature and from field tests conducted by this laboratory (CARLSON *et al.* USAFTR 6243). This hypothesis states that on exposure a readjustment in circulation occurs which leads to warmer extremities and cooler body surface, and which tends to increase the effective body weight acting as shell. It leads to the prediction that a difference should exist between those adapted and those not adapted to the cold in the rate of cooling of the hand when exposed to the cold and in the magnitude of the core. In the study to be reported the following temperatures were measured: *1*) the skin of the hand as well as body skin temperature, *2*) rectal temperature and *3*) deep muscle temperatures (25 mm. below surface) in the calf and the flexor muscles of the lower arm. Two of the subjects were adapted, the remaining 5 spent 16 to 18 hours daily outside for 14 days. Changes noted in these persons on exposure during the period were: *1*) a decreased cooling of the hand and an increased tendency to cycle in finger temperatures. This effect was noted only when the body itself was warm; an increased heat loss of the body led to rapid drop in hand temperatures. *2*) The drop in arm skin temperatures increased as drop in finger temperatures decreased. *3*) Deep leg and arm temperatures showed an increased difference from rectal temperature. Literature data are also cited to substantiate the hypothesis.

Peptidases of intestinal mucosa of ascaris lumbricoides. MARY P. CARPENTER (introduced by A. E. WOODWARD). *Dept. of Zoology, Univ. of Michigan, Ann Arbor.*

As part of a study of the hydrolases in *Ascaris*, the activity of its intestinal extracts were studied on DL-leucylglycine, DL-leucylglycylglycine, DL-alanylglycine and DL-glycylglycine using the micromethods of Linderstrøm-Lang and Holter (1931). The behavior of these peptidases compares well with those usually found. The hydrolysis of DL-LG is activated by Mn, while that of DL-LGG is not. The same enzyme preparation is approximately 2–3× as active on LGG as on LG. The fact that LGG activity has a peak at pH 7.0 (25° C.) and LG at pH 7.5 lends support to the theory that two peptidases are involved, one the Mn activated leucine aminopeptidase and the other a tripeptidase which is not metal activated. In

Ascaris the activity on AG is optimal at pH 7.5 and inhibited by cysteine. The peptidase which hydrolyzes DL-GG is particularly active and is activated by Co (0.001 M), inhibited by Zn (0.001 M), Mn (0.01 M), and cysteine (0.01 M). Preincubation with Co does not increase the activation of the enzyme, whereas similar preincubation with Mn leads to increased inhibition. The distribution of the AG and LG peptidases was studied with sections of worm alimentary canal and, as for the other hydrolases, the activity was greatest in the anterior part of the gut. The peptidases are the most active of the enzymes studied, as might be expected from the ecological niche of the worms in the pig intestine and the foods available there.

Electrometric assay of relaxin. H. R. CATCHPOLE, N. R. JOSEPH* AND M. B. ENGEL.* *Dept. of Pathology, College of Medicine; Dept. of Chemistry, College of Pharmacy; and Dept. of Dental Therapeutics, College of Dentistry, Univ. of Illinois, Chicago.*

Symphyseal relaxation in female guinea pigs has been characterized electrochemically by a study of junction potentials. In normal animals, liquid junction potentials were displaced approximately + 20 to + 30 mv. when 0.015 M NaCl was substituted for 0.15 M NaCl at a symphyseal boundary. In the highly relaxed symphyses of late pregnant animals, the displacement was around − 5 mv. These effects were correlated with the diffusion potentials of isotonic KCl. Similar techniques have been used to study relaxation produced experimentally with relaxin. Castrate female guinea pigs were primed with 1.25 μg. estradiol benzoate daily for 4 days, then received from ½ to 32 units of relaxin subcutaneously. Diffusion potentials were measured at the symphysis 8–10 hours later. Linear relations were found between observed junction potentials and the logarithm of the dose of relaxin administered. Estrogen primed animals not given relaxin showed potentials characteristic of normals. Unprimed animals showed normal potentials following the administration of 8 units of relaxin, a dose which in primed animals gives NaCl dilution potentials near zero mv. The necessity of estrogen priming in the induction of relaxation is therefore confirmed. These changes in diffusion potentials in relaxation may be interpreted in terms of a change in state of components of the ground substance of the symphysis. An electrochemical unit of relaxin may thus be defined from a given decrement (e. g. 50%) in concentration of immobile, negatively charged colloid of the symphysis, as estimated from electrochemical considerations.

Metabolism of isotopic lactate by blood-perfused isolated heart. H. MEAD CAVERT AND JOHN A. JOHNSON (introduced by W. E. PETERSEN). *Dept. of Physiology, Univ. of Minnesota, Minneapolis.*

The metabolism of isotopic lactate was studied in the completely isolated dog heart perfused with oxygenated, heparinized, autogenous blood. Sodium lactate labeled with C^{13} in the carboxyl or alpha and beta positions was injected into the perfusing blood in initial concentrations ranging from 7–18 mM/l. Respired CO_2 was collected for periods of 1.5 to 4 hours. The percentage of the total respired CO_2 derived from the administered lactate varied from 20–35%. Analysis of the glycogen of the heart tissue following the perfusion failed to reveal detectable labeling with C^{13}. These results were essentially unchanged by the following experimental variations: *1*) alteration of the blood sugar concentration by addition of glucose or glucose plus insulin, and *2*) previous administration of adrenaline to reduce the cardiac glycogen content. The finding that alpha-, beta-labeled lactate yields results almost identical to those obtained from use of carboxyl-labeled lactate indicates that the isolated heart oxidizes the 3 carbon atoms to CO_2 at essentially equal rates.

Role of the cerebellum in regulation of decerebrate spasticity in the cat. WILLIAM W. CHAMBERS AND JAMES M. SPRAGUE.* *Dept. of Anatomy, Univ. of Pennsylvania, Philadelphia.*

Acute or chronic unilateral ablation of right medial cortex of cerebellar anterior lobe facilitates decerebrate extensor spasticity in ipsilateral limbs (inhibits reciprocally contralateral), and subsequent ablation of right fastigial nucleus reverses this posture. Thus cortical lesion resembles rebound effect following stimulation of the same cortex, nuclear lesion resembles stimulation effect. These reciprocal cerebellar effects are obtained after unilateral or bilateral destruction of vestibular nerves and/or deafferentation of the limb. Tone is thus restored to a deafferented, atonic limb, by ipsilateral cortical or contralateral fastigial lesion. When, however, unilateral lesions of Deiter's and fastigial nuclei are combined, acutely or chronically, atonia results in the ipsilateral legs, equal to that following deafferentation. Bilateral chronic Deiterectomy decreases spasticity and when combined with acute removal of the fastigial nuclei abolishes it. We conclude that anterior lobe of cerebellum exerts both inhibitory and facilitatory influences on postural mechanisms. *1*) Inhibitory influence is revealed by ablation of entire anterior lobe (medial cortex and fastigial nuclei), resulting in the well-known enhancement of decerebrate spacticity. Facilitatory influence on reticular formation is evident when cerebellar ablation follows destruction of lateral vestibular nuclei, resulting in hypotonia. *2*) Inhibition and facilitation are

also revealed by unilateral ablation of medial cortex or fastigial nucleus. *3*) Decerebrate spasticity is not necessarily dependent on the stretch reflex, but to a greater extent on central mechanisms.

Effects of delayed fertilization on segmenting ova, blastocysts and fetuses in rabbit. M. C. CHANG. *Worcester Fndn. for Experimental Biology, Shrewsbury, and Dept. of Biology, Boston Univ., Boston, Mass.*

When rabbits were inseminated 2 hours after ovulation, Hammond obtained an average litter size of 0.2 young. Assuming 4 hours for transport of sperm, he concluded that fertilizability of rabbit ovum after ovulation lasted about 6 hours. Experiments were conducted to determine whether the small litter size is due to the loss of fertilizability of the ova, to the degeneration of fertilized ova before implantation, or to the failure of normal development after implantation. Insemination was performed at the time of ovulation and examined 24 hours later. There were 51% of 199 ova fertilized. In these segmenting ova, fragmentation of nucleus, fragmentation of blastomeres, and the presence of vacuoles in the blastomeres were observed. When examined 6 days after insemination, 52% of 248 ova developed into blastocysts of various sizes, but 12% of 129 blastocysts were definitely abnormal in size and in cellular structures. When insemination was performed 2 hours before ovulation and examined 24 days later, 60% of the estimated ova were either unfertilized or degenerated before implantation. Of a total 66 conceptus, 35% of normal fetuses, 6% of abnormal fetuses (exposure of brain, spinal cord or viscera), and 59% of embryonic death after implantation (mainly after the formation of fetal placenta) were observed. These observations in the rabbit are, in general, in agreement with the observations on rats and guinea pigs by Blandau, Jordan and Young.

Analysis of alcohol inhibition of the Cypridina luminescent reaction. AURIN M. CHASE. *Physiological Labs., Princeton Univ., Princeton, N. J., and Marine Biological Lab., Woods Hole, Mass.*

Reversible inhibition of the luminescent reaction of partially purified *Cypridina* luciferase and luciferin by normal alcohols, previously reported (CHASE. *Federation Proc.* 10: 25, 1951), was attributed to an inactivation of the enzyme, luciferase. These experiments have been extended and the results analyzed. The reaction was measured photoelectrically under controlled conditions in the presence of various concentrations of methanol, ethanol, *n*-propanol, *n*-butanol, *n*-pentanol and *n*-hexanol and first order velocity constants, representing enzyme activity, were calculated. These decreased, reversibly, with increasing alcohol concentration. Each additional methylene group enhanced the effectiveness of the alcohol about 2½ times. Analysis in terms of a mass law equation indicated that about 1½ alcohol molecules combined with one catalytic site to cause inactivation, although this value tended to decrease slightly with increasing molecular weight of the alcohol. For 50% inactivation of the enzyme the thermodynamic activity coefficients (mol fraction multiplied by activity coefficient) of the 6 alcohols were not constant but increased in a regular way with increasing chain length, in contrast to results from similar analyses of certain data on alcohol narcosis in living systems. The total light of the luminescent reaction, indicating the effective substrate (luciferin) concentration decreased with increasing alcohol concentration but this effect appeared to be relatively independent of the kind of alcohol present.

Competitive inhibition of Cypridina luciferase by butyl alcohol. AURIN M. CHASE AND ELIZABETH H. BRIGHAM.* *Physiological Labs., Princeton Univ., Princeton, N. J., and Marine Biological Lab., Woods Hole, Mass.*

The oxidative luminescent reaction of luciferin and luciferase, extracted and partially purified from the ostracod crustacean, *Cypridina hilgendorfii*, was studied in the presence of various concentrations of *n*-butyl alcohol. The measurements were made with a photoelectric technique, suitable either for following the entire course of the luminescent reaction or for determining only the initial velocity. The first order velocity constant of the reaction (proportional to enzyme activity) was found to be reversibly decreased in the presence of the alcohol. A 0.2 M concentration caused about 50% inactivation of the enzyme. Measurements of the initial velocity of the reaction, with and without butanol and with constant enzyme concentration and a wide range of substrate concentrations, were analyzed by the graphical method of Lineweaver and Burk (*J. Am. Chem. Soc.*, 1934). This analysis showed that the inhibition of the enzyme by the alcohol was competitive in nature. The value of the Michaelis constant calculated from the data was about 6×10^{-7} M. The calculated value of K_I, the dissociation constant of the enzyme-inhibitor complex, was about 2×10^{-1} M. If these two values be regarded as true dissociation constants (which is, of course, open to question until definitely proven) luciferase combines with butyl alcohol much more avidly than with its substrate, luciferin.

Mitochondria and tissue growth: a biochemical and morphological study. G. S. CHRISTIANSEN, BETTY DANES AND P. J. LEINFELDER (introduced by H. M. HINES). *Depts. of Ophthalmology and Physiology, College of Medicine, State Univ. of Iowa, Iowa City.*

A correlation has been made between the morphology of cellular growth and the metabolic activity which characterizes this growth. Embryonic chick heart tissue was grown in isolated culture by a combination of new techniques which allows periodic morphological observation by phase microscopy and continuous observation of respiration of the same piece of growing tissue. Observations were made on control cultures and on cyanide inhibited cultures initially and after 12, 24, 36, 48 and 72 hours of growth. Growth rates were measured by count of total migrated cells per culture; respiratory rate was measured as Q_{O_2} per cell. Morphological observations included measurement of mitotic index, count of aberant nuclear forms, estimate of the lipid content of the cells (based on the number and size of fat droplets), and observation of the number, shape and general appearance of the mitochondria. The biochemical site of cyanide inhibition has been shown to be in the oxidative enzymes of the mitochondria. And the most striking morphological effect of low concentrations of cyanide is also on the mitochondria. In the range from 10^{-7} to 10^{-5} M the normally filamentous mitochondria are progressively caused to coil, become granular, and finally clump together at the cell membrane. In the same concentration range the growth is first stimulated then is sharply inhibited; mitosis is almost completely inhibited; the lipid content increases; and the respiration is inhibited proportional to the logarithm of the cyanide concentration.

Pneumatic refractometer—a continuous fast recording gas analyzer. HANS G. CLAMANN (introduced by OTIS O. BENSON, JR.). *USAF School of Aviation Medicine, Randolph Field, Tex.*

A new principle has been applied to obtain fast recordings of changes in composition of gas mixtures as they occur in respiration. This principle is based on the fact that the refractive index (r.i.) of a gas is proportional to its density, or in a mixture of gases with different r.i. to its concentration. To obtain refraction of a light beam, the gas to be analyzed has to form a plane surface to a reference gas of another optical density. A constant flow of the reference gas through the analyzing cell in the shape of a Venturi tube provides suction to a slit-like jet nozzle. The analyzed gas then enters the cell through this nozzle in form of a well defined small band, imbedded in the reference gas. A light beam from a single filament lamp traverses the analyzing cell, directed by 2 cylindric lenses as windows, and finally forms an image on the screen. The light beam passing from the reference gas into the analyzed gas is refracted and therefore more or less spread out, thus changing the brightness of the image. These changes are transmitted by photocell to a recording device. In respiration studies, concentration of carbon dioxide can be measured during a single breath whereby the speed of measurement is limited mainly by the latency interval due to the dead space of the connecting tubing. The principle is not limited to carbon dioxide, but applicable to all gases, depending upon their optical qualities.

Effects of 20,000 ft. simulated altitude on myoglobin content of animals with and without exercise. ROBERT T. CLARK, JR., DOMINIC CRISCUOLO AND CHARLES K. COULSON (introduced by ULRICH C. LUFT). *USAF School of Aviation Medicine, Randolph Field, Tex.*

In one group of experiments, heart, gastrocnemius, and diaphragm muscles of hamsters were analyzed for myoglobin content after a continuous acclimatization period of 6 weeks without exercise to simulated altitude of 20,000 ft. Results (mg./gm/wet tissue) are as follows: acclimatized, heart muscle, 11.2; gastrocnemius, 9.7; diaphragm, 10.0; controls, heart, 12.3; gastrocnemius, 10.4; diaphragm, 10.2. Hemoglobin (gm.) showed for acclimatized group 18.8, controls, 12.0. In the second group, the gastrocnemius was removed from one leg before acclimatization and from the other leg at the end of the exposure period. Results for myoglobin are as follows: acclimatized, 9.7; controls, 10.2. In another series, 100 male rats were divided into 4 groups. *Groups I* and *II* were exposed to a simulated altitude of 20,000 ft. for 6 weeks. In addition, *Group I* was exercised on a treadmill at altitude for 30 minutes each day. *Groups III* and *IV* were kept at ground level. *Group III* was exercised on treadmill for 30 minutes each day. The results show approximately 200% increase of myoglobin in heart muscle for animals exercised during acclimatization and 50% increases or diaphragm and gastrocnemius muscle as compared with animals exercised at ground level. *Groups II* and *IV* had lower myoglobin levels than other groups.

Localization of stretch reflex. LEONARD A. COHEN (introduced by JOHN F. FULTON). *Lab. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.*

The localization of the stretch reflex in quadriceps femoris muscle was investigated in decerebrated cats. A small longitudinal strip of quadriceps muscle, about 3 min. in diameter, was stretched and its resulting reflex tension was measured. The concomitant reflex tension developed in the rest of quadriceps was separately measured. A comparison of the two responses showed the localization of the stretch reflex. Tensions were measured by a new type of movable anode transducer. When a 3 mm. stretch was applied to a muscle strip, the reflex response was usually confined completely to the strip, the rest of quadriceps showing no response. Under condi-

tions of increased cord activity the same 3 mm. stretch stimulus produced a spread of the reflex response to include the rest of the muscle as well as the small strip which was stretched. The data were analyzed and developed to show that a dual mechanism is involved in stretch reflex localization. A preferential, very highly localized pathway exists which can fire at low levels of quadriceps motoneuron pool activity. This pathway allows almost completely independent adjustments to stretch of muscle units at least as small as 3 mm. in diameter. At higher levels of quadriceps motoneuron pool activity a secondary, more diffuse system is activated which can produce stronger, less finely localized responses to stretch. (Aided by a grant from the George H. Knight Memorial Fund, Yale Univ. School of Medicine.)

Changes in maximum diffusing capacity of lungs with age. JEROME E. COHN (introduced by JOSEPH L. LILLIENTHAL, JR.). *Dept. of Environmental Medicine, Johns Hopkins Univ., Baltimore, Md.*

The maximal diffusing capacity of the lungs was estimated for 15 normal males between 29 and 76 years of age. These studies were undertaken to explore the potentialities of the maximal diffusing capacity as related to the size of the pulmonary capillary bed. The reasons for postulating such a relationship are presented elsewhere by Riley. A linear relationship was observed between maximal diffusing capacity and age. The values scattered about a straight line connecting 40 ml/min/mm.Hg gradient/sq.m.BSA at age 29 and 15 at age 70. The statistical correlation coefficient for these data is 0.881 ($P < .01$). On the basis of empirically established criteria it is believed that the values obtained represent maximal or near maximal values. The decrease in maximal diffusing capacity with age probably indicates, to a first approximation, a corresponding decrease in the size of the pulmonary capillary bed with age.

Effects of carbonic anhydrase inhibitor #6063 on electrolyte concentration in canine gastric juice. HENRY COLCHER,* HENRY D. JANOWITZ AND FRANKLIN HOLLANDER. *Gastroenterology Research Lab., Mount Sinai Hospital, Research Service Columbia Univ. Div., Goldwater Memorial Hospital, and the Dept. of Medicine, College of Physicians and Surgeons, Columbia Univ., New York City.*

Berliner *et al.* have reported striking rises in urinary output of Na and K during inhibition of acid formation following injection of 2-acetylamino-1,3,4-thiadiazole-5-sulfonamid (compound #6063), a potent carbonic anhydrase inhibitor *in vitro*. We are reporting separately (see abstract, JANOWITZ, COLCHER AND HOLLANDER) that intravenous injection of this compound markedly inhibits histamine-induced gastric secretion in Heidenhain pouch dogs. The present report describes the variations in concentration of Na, K, Cl and acidity observed in histamine-induced secretion (repeated injection every 10 minutes) with and without inhibition with #6063. In 24 experiments (4 dogs) there was good inverse correlation between Na concentration (range: 3.5–144 mN) and acidity (10–149 mN), with and without inhibition. However, this did not obtain for K concentration (range 0.8–12.8 mN) or for output; highest concentrations occurred regularly in the earliest specimens following histamine stimulation, and were followed by a steady decrease regardless of acid formation. The changes in concentration were similar to those observed with single histamine injection experiments without #6063 (to be reported elsewhere). This pattern for K in gastric juice following the injection of #6063 is in striking contrast to that reported for the kidney. The implications of these observations will be discussed. (Acknowledgment is made to Dr. R. O. Roblin Jr., American Cyanamid Company, for generous supplies of compound #6063.)

Protective effect of spleen homogenates administered to mice after X-irradiation. LEONARD J. COLE, MARIE E. ELLIS, MAURICE C. FISHLER AND VICTOR P. BOND (introduced by GEORGE A. FEIGEN). *Biological and Med. Sciences Div., U. S. Naval Radiological Defense Lab., San Francisco, Calif.*

Experimental evidence involving post-irradiation protection of mice by means of spleen implants and spleen shielding suggests the presence of a splenic factor affecting recovery of mice from X-irradiation (JACOBSON *et al. Science* 113: 510, 1951). In an attempt to ascertain the nature of this factor, i.e. whether humoral or cellular, a study was made of the protective effect in X-irradiated mice afforded by spleen homogenates, in which the integrity of the majority of spleen cells had been disrupted by homogenization. Spleens from mice (1–3 weeks old) were homogenized with M/15 phosphate buffer (pH 7.2) in a motor-driven homogenizer with ground glass surfaces. The mortality of adult LAf_1 mice receiving 650r or 700r single dose whole body X-irradiation was either prevented or markedly reduced after i.p. administration of such homogenates approximately 1 hour after X-irradiation. Of 23 control mice exposed to 650r in one typical experiment, 68% were dead at the conclusion of the 30-day observation period, whereas none of the group of 19 mice receiving spleen homogenate (equivalent to 1 spleen per mouse) died. The radiation protection afforded by spleen homogenate injection was likewise reflected in the minimal body weight losses observed in the treated mice as compared with the control animals. The supernatant obtained by centrifugation of the whole

spleen homogenate at 2000 rpm for 7 minutes, was devoid of protective activity, whereas the sediment still exhibited activity. Aqueous spleen extracts prepared at various pH conditions and solute concentrations, were uniformly lacking in protective activity. The relationship between these and other experimental data, as related to the problem of spleen protection in injury resulting from ionizing radiations, will be discussed.

Effect of diet and ACTH on nucleic acid metabolism in the rat. JOHN A. D. COOPER (introduced by WILLIAM B. BRADLEY). *Dept. of Biochemistry, Northwestern Univ. Med. School, Chicago, Ill.*

The effect of low protein diet and ACTH on the nucleic acid metabolism in the liver, spleen, kidney and intestine have been studied in the rat. The nucleic acid phosphorus content and the incorporation of radioactive phosphorus into these fractions have been estimated by a modification of the method of Schmidt and Thannhauser. No significant change from control animals was found in the concentration of ribonucleic acid or desoxyribonucleic acid phosphorus in tissues from animals on the low protein diet. There was an increase in the rate of incorporation of radiophosphorus into the ribonucleic acid phosphorus fraction in the liver. Following ACTH the RNAP content of all organs studied increased. There was no change in the DNAP content or the rate of incorporation of radiophosphorus into either fraction. (Work performed under contract with the Atomic Energy Commission.)

Bleeding time and clot resistance studies in mice, rats and rabbits following whole body X-irradiation. ALFRED L. COPLEY. *New York Med. College, New York City and Marine Biological Lab., Woods Hole, Mass.*

Bleeding time tests into physiological saline at 37.5°C. were developed, using the rat's tail and the rabbit's paw. In 66 normal rats 154 bleeding times were within 3 minutes, as previously reported in human subjects and mice (A. L. COPLEY AND J. J. LALICH, *J. Clin. Investigation* 21: 145, 1942; *Am. J. Physiol.* 135: 547, 1942). In 37 normal rabbits 75 bleeding times ranged from 20 to 270 seconds, although 90% of these values were under 180 seconds. Prolonged bleeding times of 9 minutes or more were mainly found after the 6th day following total body exposure to ionizing X-irradiation in the lethal range. Clot resistance is a hemo-rheological *in vivo* test (A. L. COPLEY, *J. Colloid Sci.*, in press) which estimates the firmness of a clot (wound thrombus) and its ability to adhere to the inflicted vessel wounds. Cuff-pressures, below the systolic pressure values, applied proximally to the wound 5 and 20 minutes after the bleeding time tests, resulted in renewal of bleeding (decreased clot resistance), indicating impaired hemostasis. This bleeding test in rats and rabbits was based on our standard procedures previously reported in men and mice. Decreased clot resistance was observed in rabbits, mice, and infant and adult rats within 24 hours following exposure to dosages of radiation varying from 700 to 2100 r, the larger dosages producing higher frequency. Clot resistance tests may prove to be useful in early detection of radiation injury in men. (Work performed under Contract No. AT(30-1)-780 of the Atomic Energy Commission and Contract No. N7onr-40102 of the Office of Naval Research, Department of the Navy.)

Antagonistic effects of calcium ions and heparin on gelation phase of blood coagulation. ALFRED L. COPLEY. *New York Med. College, New York City and Marine Biological Lab., Woods Hole, Mass.*

Blood systems containing enough heparin to increase markedly the gelation time without rendering blood incoagulable best demonstrate the antagonistic effect of Ca^{++} on heparin action. Amounts of Ca^{++} varying from 3.3 gamma to 0.54 mg. produced the accelerating effect when added to 1 cc. of 50% blood in physiological saline containing 0.01 mg. heparin. Larger concentrations of Ca^{++} did not decrease but augmented the anticoagulant effects of heparin. The gelation inhibiting effect of heparin also is found in thrombin-fibrinogen systems. To 5% fibrinogen (0.2 cc.) was added 50 N.I.H. units of thrombin (0.1 cc.) resulting in firm gel formation. Addition of 0.5 mg. heparin to these thrombin-fibrinogen systems did not significantly delay fibrin precipitation but markedly inhibited gelation. These findings indicate that heparin inhibits the third or gelation phase of blood coagulation (A. L. COPLEY, *Federation Proc.* 10: 29, 1951; *Am. J. Physiol.*, in press). The antagonistic action of Ca^{++} to heparin in the gelation phase becomes evident in fibrinogen-heparin-thrombin systems. Ca^{++} is thus shown to promote gelation like 'geloplastin' (A. L. COPLEY, *J. Colloid Sci.*, in press), an accelerating factor found in tissue juice and plasma. Very high $CaCl_2$ concentrations, of 0.108 M (0.05 cc.) or more, however, are not antagonistic to heparin and result in significant prolongation of fibrin precipitation times and more pronounced inhibition of fibrin gelation. Systems containing heparin always produced soft gels. (Work performed under Contract No. N7onr-40102 of the Office of Naval Research, Department of the Navy.)

Capillary-tissue exchange of non-metabolized substances studied as a function of volume perfused. REUBEN COPPERMAN (introduced by SEYMOUR S. KETY). *Dept. of Physiology and*

Pharmacology, Graduate School of Medicine, Univ. of Pennsylvania, Philadelphia.

In an attempt to examine the processes involved in transcapillary exchange of non-metabolized substances, the hindlegs of a bullfrog were perfused by the technique of Läwen and Trendelenburg using a frog Ringer's solution containing bovine albumin and a diffusible non-metabolized substance, NaSCN. The number of variables was reduced by perfusing a nearly homogeneous tissue mass at constant pressure and concentration. Venous outflow was collected continuously in successive samples and their volume and thiocyanate concentration were determined. Venous concentration was then plotted against cumulative perfusion volume instead of time. It was found that, when plotted in this manner, all venous concentration curves tended to have similar characteristics. In 5 frogs perfused over a wide range of constant pressures, superimposable curves were obtained when concentration was plotted against cumulative perfusion volume/total SCN space; this takes into account variations in the total SCN space among frogs. This result has significant implications as regards a filtration or diffusion limited model of transcapillary exchange.

Responses of fetal rat heart to drug administration in the mother. E. L. Corey and C. R. French.* *Univ. of Virginia Med. School, Charlottesville.*

Simultaneous E.C.G. records were obtained from both mother and fetus (0.215–5.736 gm.) without removing the latter from the uterus and while maintaining apparently normal circulatory relationships. The fetal heart rate averaged 46.1% of that of the mother in 50 individuals, while E.M.F. of the fetal heart approximated 75% of that of the adult, even in the youngest (C-R length 11 mm.) animals studied. High resistance of the fetal heart to anoxia was apparent following maternal death from hemorrhage or anesthesia. Thus, fetal cardiac activity was found to cease (av.) 57.9 min. after the maternal heart had stopped beating, and 69.1 min. following cessation of respiratory activity in the mother. The intravenous injection of acetylcholine in the mother produced a slight, but definite, retardation of the fetal heart, but even prolonged and repeated stimulation of the maternal vagus failed to result in any (statistically) significant effect. No positive response of the fetal heart to either adrenalin or atropine in the maternal circulation could be demonstrated, other than a slight depression following massive doses. Caffeine and alcohol in the maternal blood stream affected fetal cardiac activity similarly, but to a lesser degree than in the adult. Placental transmission to the former drug appeared to be quite slow (av. 31 min.), on the basis of E.C.G. records obtained.

Renal sodium reabsorption in dogs under conditions of loading with various sodium salts. Samuel A. Corson, Elizabeth O'Leary Corson* and Abraham L. Siegel.* *Kirksville College of Osteopathy and Surgery, Kirksville, Mo.*

Hypertonic solutions (1600 mEq/l.) of sodium chloride, acetate, succinate and fumarate were infused into anesthetized dogs at the rate of 1.6 ml/minute. Pre- and postexperimental controls were obtained by the infusion at the same rate of 0.16 M NaCl. Glomerular filtration rate was measured by exogenous creatinine clearance. Sodium was determined by means of a flame photometer. Under all conditions of loading with NaCl or with the sodium salts of organic acids the quantity of sodium reabsorbed/unit of glomerular filtrate remained constant, averaging 137 mEq/l. of glomerular filtrate for the pre-experimental controls; 137.5 mEq/l. during the infusion of the hypertonic solutions; and 140 mEq/l. for the postexperimental control periods. (Supported in part by a research grant from the U. S. Public Health Service).

Inulin and chloride space in muscle. Ernest Cotlove (introduced by Bert R. Boone). *Sec. on Kidney and Electrolyte Metabolism, Natl. Heart Inst., Natl. Insts. of Health, Bethesda, Md.*

The volume of distribution of inulin in muscle has been examined as a function of the time of equilibration and compared with the simultaneously measured chloride space. Using a technique developed in this laboratory, unanesthetized rats have been given constant intravenous infusions of inulin for periods varying from 1–15 hours. Under these conditions, the glomerular filtration rate remained within normal limits and the plasma inulin level was maintained constant throughout the infusion, thus allowing an approach to equilibration. Inulin and chloride were analyzed in a heated water extract of an homogenate of the large muscles of both legs, thus avoiding variation due to sampling. Inulin was determined by an alkali method modified from that of Ross and Mokotoff (*J. Biol. Chem.* 190: 659, 1951). The inulin blank in muscle was evaluated from recovery experiments using muscle from uninfused rats. The blank was found to be fairly constant, the variation in the blank representing less than 5% of the total inulin in muscle. In a series of 30 experiments which included infusion periods of 1, 2, 6 and 15 hours, the inulin space showed a progressive approximation to the chloride space with increasing time of equilibration. The regression line relating the ratio of inulin/chloride space to time of infusion showed a highly significant slope, with P less than 0.001.

Changes in extracellular space induced by intravenous saline solution in man. BETTY CRAWFORD* AND MARIO GAUDINO. *Depts. of Anesthesiology, Surgery and Physiology, New York Univ. College of Medicine, New York City.*

Extracellular space was measured by means of inulin. Both the total recovery and the calculation-by-difference methods were used, the dual procedure permitting the determination of the inulin space before and after saline infusion and affording a check on the latter by the total recovery method. The time required for uniform distribution of inulin (2 or more spaces agreeing within 5%) was reduced by using an accelerated infusion as a priming injection. After uniform distribution, 2 or 3 l. of isotonic saline solution were infused at a rapid rate (40–60 cc/min.), and space measurements were repeated at 1 and 2 hours. Twenty observations were made in 18 clinically normal subjects. In 17 of the 20 tests, the inulin space 1 hour after the end of the saline infusion was larger than the control value; in the remaining 3 the change was negligible. The increase was less than the volume of saline solution administered in 6 tests, and approximately equal in 4, while in 7 tests the inulin space increased by 1–3.8 l. more than the volume of saline solution administered. Of 6 patients studied again on the second hour after the end of the infusion, 2 showed no substantial change in inulin space, 2 showed a further increase, and 2 showed a reduction. Since plasma sodium scarcely changed, it is clear that sodium and water can move into and out of the cells with surprising rapidity.

Interaction between certain nerve blocking drugs and sodium ions. FREDERICK CRESCITELLI. *Dept. of Zoology, Univ. of California, Los Angeles.*

The results of 3 different types of experiments on desheathed sciatic nerves of bullfrogs suggest that nerve block produced by certain drugs involves an interference with a sodium mechanism. The three types of experiments and the results are enumerated: *1)* The *A* fiber activity decreased rapidly and disappeared in 2 to 15 minutes when a segment of the nerve was treated with a solution lacking NaCl. Recovery occurred when the segment was immersed in a 0.011 NaCl solution. Immersion of the segment in a NaCl free solution which contained an ordinarily ineffective concentration (in Ringer's solution) of cocaine, benadryl, eserine or DFP caused conduction block at a rate which was significantly faster than with the sodium free solution without the drug. Pretreatment of the segment with one of these drugs inhibited the recovery of activity with the usually effective 0.011 M NaCl solution. *2)* Whereas concentrations of amyl carbamate or of cocaine could be made up in Ringer's solution which had little or no blocking action, these same concentrations, when made up in 0.022 M NaCl, caused rapid and reversible block. The 0.022 M NaCl of itself produced no block. *3)* The conduction block produced by certain concentrations of amyl carbamate, cocaine, *d*-amidone or *l*-amidone, when made up in 0.022 M NaCl, was rapidly and completely reversed by the corresponding solution of the drug at the same concentration as above but made up in 0.11 M NaCl. Lithium was able to replace sodium in many of these reactions. All the solutions were isotonic with the nerve fibers. In NaCl deficient solutions the osmotic deficit was made up by adding sucrose, choline chloride or tetramethyl ammonium bromide.

Response of dog's pancreas to secretin during periods of induced hyperglycemia. J. O. CRIDER AND J. E. THOMAS. *Dept. of Physiology, Jefferson Med. College, Philadelphia, Pa.*

Babkin (1944) as well as LaBarre *et al.* (1928–1933) have reported that hypoglycemia decreases and hyperglycemia increases the enzyme output from the pancreas. Results with a contrary implication obtained by us (1947) prompted the present study. Volume and specific gravity of pancreatic juice secreted by chronic fistula dogs were determined during the continuous intravenous infusion of secretin alone or accompanied by normal saline or 5% glucose. Twenty-one experiments were performed on 3 dogs; in 17 of the experiments the blood sugar was increased by administration of glucose. Fasting blood sugars averaged 97 mg.%; samples collected during glucose administration averaged 149 mg.%; the average of the maximum values obtained in each experiment was 162.9 mg.%. In 3 experiments blood sugars rose above 200 mg.%. No change in either volume or specific gravity of the juice was observed that could be attributed to the injection of glucose or to comparable amounts of normal saline. We conclude that neither moderate hydration nor a considerable increase in blood sugar modifies the response of the dog's pancreas to secretin.

Blood volume in the hypothermic dog. HENRY D'AMATO* AND A. H. HEGNAUER. *Boston Univ. School of Medicine, Boston, Mass.*

In nembutalized dogs chilled to 20° by immersion in an iced bath, circulating plasma (PV) and erythrocyte (RV) volumes were measured with T 1824 and P^{32} tagged cells respectively, and hematocrits (Ht) by spinning at 3000 rpm for 30 min. Mixing of both dye and tagged cells was found complete at 45 min. (normal temperature) and 45–60 min. (20°). Plasma protein concentrations were computed from specific gravity as measured against Cu SO_4 or refractometrically. Differences from normal noted at 20° follow:, RV, $-2.6\% \pm 2.1$; PV, $-12.8\% \pm 1.5$; plasma

protein conc., −1.7% ± 1.1; Ht, +15.2% ± 1.7. Previous studies on tissue water (muscle, liver, heart) in dogs chilled to the same temperature showed no measurable increases at expense of plasma. An apparent increase in RV of 18–33% obtains at 20° when calculated from PV and Ht. It thus appears that neither specific gravity nor Ht are reliable indices of blood volume change in hypothermia. The data suggest rather that erythrocytes and some 87% of the plasma are confined to a smaller than normal vascular bed, with some 13% of the plasma sequestered in vessels from which erythrocytes are excluded.

Pituitary-thyroid-adrenal function in the goitrous guinea pig. SAVINO A. D'ANGELO. *Dept. of Anatomy, Jefferson Med. College, Philadelphia, Pa.*

The chronic administration of propylthiouracil (0.1% in the ration) to female guinea pigs induced pronounced histo-physiologic change in thyroid and hypophysis without apparent change in adrenocortical function. Weights and ascorbic acid concentrations of adrenals remained within normal limits throughout 160 days of treatment despite thyroid hyperplasia and pituitary enlargement. Thyrotrophic hormone (TSH) assays (stasis tadpole technic) of blood and anterior pituitary in drugged animals disclosed extreme shifts in the balanced hormonal relationship between thyroid and hypophysis. Periods of goitrogen treatment too short (7 days) to effect anatomic change in the thyroid resulted in lowered pituitary TSH content with concomitant rise in blood hormone levels. With longer treatment thyroid size and pituitary thyrotrophin content increased progressively, whereas blood TSH titers remain relatively constant. TSH concentrations in the pituitary were approximately 200 and 800% above normal in animals maintained on the drug for 40 and 78 days respectively. Assay of pituitary adrenocorticotrophin after prolonged treatment (adrenal ascorbic acid depletion method) indicated essentially normal concentrations of this trophic principle. It is concluded tentatively that in the goitrous guinea pig anterior pituitary-adrenocortical interaction is not appreciably disturbed and that augmented TSH production by the pituitary is required to sustain the elevated blood TSH level essential for thyroid hyperplasia. (Supported in part by grant RG-2407, National Institutes of Health, U. S. Public Health Service.)

Energy cost of load carrying on a treadmill. FARRINGTON DANIELS, JR., JAN H. VANDERBIE AND C. LAWRENCE BOMMARITO (introduced by EARL R. LOEW). *Quartermaster Climatic Research Lab., Lawrence, Mass.*

The energy cost of carrying pack loads of 28 to 80 lb. was studied on a motor-driven treadmill at 3.5 mph. The subjects weighed from 144 to 198 lb. Load distributions high on the back, low on the back, and around the waist were employed. For the high back pack the energy cost in Cal/m²/hr. for 6 subjects averaged 181 for 31 lb., 194 for 46 lb., 212 for 61 lb., and 242 for 76 lb. The average cost for walking without load under the same conditions was 161 Cal/m²/hr. The relationship between load and metabolic cost with the high back carry is expressed by: Cal/m²/hr. = 161.7 + .1994 (pounds load) + .0110 (pounds load)². The energy cost for the low back pack averaged 178, 195, 214 and 235 for the same weights. Energy cost of carrying a load distributed around the waist averaged 192 for 28 lb., 208 for 43 lb., 227 for 62 lb., and 235 for 80 lb. The energy cost of walking at 3.5 mph with a 46-lb. load was found to be 8% greater on a level road than on a horizontal treadmill.

Effect of chloretone on oxygen consumption and negative spike response of cerebral cortex. P. W. DAVIES. *Dept. of Biophysics, Johns Hopkins Univ., Baltimore, Md.*

A significant improvement in the technique of local perfusion of cat cerebral cortex has been made. This is accomplished by the addition of fresh washed red blood cells from the same animal to the solutions previously used (Krebs solution containing 6 mM glucose and 2% gelatin). The inadequacy of the solutions not containing cells was more and more clearly seen when the initial negative spike response to direct local electrical stimulation was used as a criterion of a healthy preparation. Whereas with the old solutions the negative spike dwindled steadily to a low value in an hour's time, the solution containing red blood cells is capable of maintaining the response virtually undiminished for as long as 3 hours. Whether this is due to improved oxygenation, or to some factor washed down with the cells, has not been established at present. The improved perfusion fluid has been used in the study of the effect of chloretone on surface oxygen consumption (measured with an occlusion type oxygen cathode), and on the negative spike. If Chang's interpretation is correct, the spike also arises in the surface layer of the cortex. When the concentration of this narcotic is 2 mM, the rate of oxygen consumption diminishes slightly, and the action potential seems little affected if at all. At 5 mM, on the other hand, the consumption is reduced by a factor of 2, and the spike is virtually abolished, though it recovers rapidly when the chloretone is washed out.

Velocity and amplitude of microphonic response of guinea pig cochlea. H. DAVIS, I. TASAKI* AND J. P. LEGOUIX.* *Central Inst. for the Deaf, St. Louis, Mo.*

Three pairs of nichrome steel electrodes (20 μ) were inserted into the basal, the second, the third and/or the apical turns of the cochlea of guinea pig. One electrode of each pair was in scala tympani, the other in scala vestibuli. Each response was nearly pure cochlear microphonic generated by a segment of basilar membrane about 1 mm. long. The voltages and the time relations of the microphonic responses to pure tones and tone pips were measured. Low tones evoke microphonics from all 4 turns, but as the frequency is increased (holding the response from the basal turn constant) first the apical turn, then the third turn and then the second turn cease to respond. The diminution in response at any one position is quite abrupt, and the curve shows approximately exponential decay as a function of frequency. Frequency analysis as a function of position along the membrane is very clear, and the space pattern for each frequency is a long slowly-rising plateau with abrupt termination. The space-time pattern of an acoustic signal is a traveling wave which requires only 0.03 msec. to traverse 2 mm. in the basal turn, 0.20 msec. to reach turn II (11 mm.), 1.0 msec. to reach turn III (15 mm.), and 2.5 msec. to reach the apical turn (17.5 mm. from round window, 1.0 mm. from helicotrema). The velocity thus falls from about 70 m/sec. to about 2 m/sec. These velocities are in good agreement with the observations of Békésy and the calculations of Fletcher and others. (Work done under contract N6onr-272 with Office of Naval Research.)

Comparison of insulin sensitivity of adrenalectomized and hypophysectomized dogs. R. C. DE BODO, M. W. SINKOFF* AND S. P. KIANG.* *Dept. of Pharmacology, New York Univ. College of Medicine, New York City.*

The insulin sensitivity of hypophysectomized dogs can be abolished either with growth hormone or ACTH. To determine the role of these hormones as physiological anti-insulin agents, the insulin sensitivity of adrenalectomized and of hypophysectomized dogs was compared. The insulin sensitivity of 5 adrenalectomized dogs was determined 7–214 days postoperatively. In each dog, the insulin sensitivity was tested while on maintenance doses of desoxycorticosterone acetate (DCA) and then tested repeatedly during a DCA-free period (lasting 8–14 days) until signs and symptoms of severe adrenal insufficiency appeared. These dogs were subjected on several occasions to such DCA-free experimental periods. All adrenalectomized dogs, while on DCA maintenance, showed a normal response to the test dose of insulin (0.025 μ/kg. i.v.). When off DCA and still eating well, 2 dogs (in the postabsorptive state) exhibited an insulin sensitivity greater than normal but apparently less than that of the hypophysectomized dog; this sensitivity did not increase with the duration of the DCA-free period even when these animals showed signs of severe adrenal insufficiency. When off DCA and eating well, the remaining three dogs manifested a normal response to insulin. They became sensitive only when they had fasted for more than 40 hours, but the sensitivity again seemed to be less than that of the hypophysectomized dog. Conclusions: The adrenal steroid-free, untreated, fed adrenalectomized dog is either not sensitive to insulin or less sensitive than the hypophysectomized dog. The adrenal cortical atrophy as a factor in the insulin hypersensitivity of hypophysectomized dogs will be discussed.

Effect of ACTH on carbohydrate metabolism in hypophysectomized dogs. R. C. DE BODO, M. KURTZ,* N. LANE* AND S. P. KIANG.* *Dept. of Pharmacology, New York Univ. College of Medicine, New York City.*

Seven 'long-term' hypophysectomized dogs, exhibiting a high degree of sensitivity to insulin and a marked resistance to the hyperglycemic action of adrenaline, were started on ACTH regimens between 85 and 570 days postoperatively. Several ACTH preparations, having potencies of 3–20 IU/mg., prepared by R. W. Bates (E. R. Squibb & Sons) were used. The total daily dosage of ACTH, which varied from 3–35 IU/kg., was divided equally and given intramuscularly at 9 A.M., 1 P.M., 5 P.M. and 10 P.M. Administration of ACTH was continued for a period of 23–125 days. In every animal the ACTH exerted a very potent anti-insulin effect, i.e. it initially diminished the exaggerated response to insulin and subsequently abolished it; in some animals the response to insulin became less than normal. Concurrently, the hyperglycemic response to adrenaline increased and became even greater than that which is observed in normal dogs. Eventually, the ACTH treatment caused slight impairment in the tolerance to intravenous glucose. Regeneration of the adrenal cortex occurred in all animals as evidenced by changes in the width of the cortex, size of the cells and cytoplasmic vacuolization. An excess of collagenous connective tissue still present throughout the cortex after ACTH treatment is considered evidence that generalized adrenocortical atrophy existed prior to ACTH treatment. The weight of the two adrenal glands in 4 of the animals was within normal range. Conclusions: prolonged administration of large doses of ACTH, perhaps by producing hyperadrenocorticalism, is able to abolish the insulin hypersensitivity and resistance to the hyperglycemic action of adrenaline of hypophysectomized dogs.

Comparative cardiovascular effects of nor-epinephrine, *l*-epinephrine and commercial epinephrine. B. DEBOER AND H. E. EDERSTROM. *Depts. of Physiology and Pharmacology, St. Louis Univ. School of Medicine, St. Louis, Mo.*

Cardiovascular responses following intravenous and intra-arterial administration of nor-epinephrine, *l*-epinephrine, and commercial epinephrine were observed in dogs anesthetized with pentobarbital. Blood pressure was recorded optically, and vasomotor responses measured from recordings of tissue pulse amplitudes in skin of hind foot and tongue with a photoelectric plethysmograph. 'Equipressor' doses of commercial epinephrine 25, *l*-epinephrine 19, and nor-epinephrine 15 γ/kg. were used. Nor-epinephrine and *l*-epinephrine intravenously gave about 50% rise in systolic blood pressure, slightly constricted foot skin vessels, but dilated tongue vessels. Commercial epinephrine had less pressor effect, slightly dilated tongue vessels, but markedly constricted foot pad vessels. In the same doses, all 3 drugs via femoral artery had marked and almost maximal constrictor effects on foot pad vessels but produced little change in blood pressure. Injections into the carotid artery disclosed moderate constricting action on the tongue by nor-epinephrine, but not with *l*-epinephrine and commercial epinephrine. By this route nor-epinephrine gave about 40% rise in blood pressure, while the other drugs gave about 20% rise. Intravenously, all drugs had typical epinephrine-like actions on the heart. An increase in rate and force of contractions was followed by reflex bradycardia. Given intra-arterially, the drugs had less cardiac and pressor effect; probably because of the slow rate of discharge from the highly constricted vessels into the general circulation.

Effects of starvation on placentoma reaction in the rat. VINCENT J. DEFEO* AND IRVING ROTHSCHILD. *Dept. of Physiology, Ohio State Univ-College of Medicine, Columbus.*

Acute starvation of adult, intact female rats resulted in marked reduction in the percentage of rats forming placentomata and in placentoma size, following appropriate uterine traumatization on the 4th, and examination by autopsy on the 9th day of pseudopregnancy. No placentomata formed in rats starved for the entire 9 day period. When starvation occupied days 1 through 4 or 4 through 9, inclusive, of pseudopregnancy about half the animals formed placentomata that were considerably smaller than those appearing in non-starved pseudopregnant controls. Thus decreased luteal activity or reduced uterine responsiveness or both, may be the consequences of starvation. To test at least one of these possibilities, progesterone was administered daily for 8 days to estrogen primed long term castrates subjected to the same starvation and traumatization procedures as were the intact pseudopregnant rats. Placentomata formed in all progesterone treated rats. Their size was, in general, directly related to the daily dose of progesterone (1.0 or 2.0 mg.), and bore no relation to the length of the starvation period. However, all 3 periods of starvation apparently resulted in a slight reduction of placentoma size as compared with those produced by non-starved, but similarly progesterone treated long term castrate controls. It would thus appear that starvation interferes primarily with the pituitary-corpus luteum system, and secondarily (and only to a minor degree) with the progesterone-uterus system, in the placentoma reaction. The exact site of interference and its mechanism is under investigation.

Relationship between superficial and hidden motor cortex in unanesthetized cat. José M. R. DELGADO (introduced by ROBERT B. LIVINGSTON). *Lab. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.*

Multilead electrodes (up to 30) have been implanted for periods of as long as a year on the surface of the cerebral cortex and in the grey matter of the sulci of the frontal lobes. The functions of the superficial motor cortex located in the anterior and posterior sigmoid giri, and also the functions of the hidden motor cortex situated within the cruciate and presylvian sulci, were studied simultaneously in the unanesthetized cat. Both of these areas play functionally similar roles in the control of motor activity. It was determined that each area has a complete representation of body musculature. The thresholds and the types of motor responses elicited were found to be similar in both areas. The excitability of the superficial and hidden motor cortex varied in accordance with the activity of the animal, thresholds becoming higher during sleep and lower when the animal was active. When the two regions under consideration were stimulated simultaneously summation of excitation or inhibition resulted. However, the arrest reaction and the food ingestion response were obtained only from the hidden cortex. Responses from the hidden motor cortex were evoked even after the destruction of the superficial motor cortex and degeneration of its pathways. Chemical stimulation with acetylcholine of the hidden cortex in the unanesthetized cat also elicited motor responses.

Effects of cortisone on muscular contraction. E. C. DEL POZO AND J. NEGRETE.* *Inst. de Salubridad y Enfermedades Tropicales, Mexico, D.F.*

In despite of the fact that the muscular effects of cortisone have been the physiological basis for the discovery and assay of its activity (INGLE, D.

J. J. *Clin. Endocrinol.* 10: 1312, 1950), no systematic study of cortisone on muscular contraction has been reported. Ingle *et al.* (*Am. J. Physiol.* 157: 99, 1940) have found that previous adrenalectomy was required for the detection of the effect of adrenal cortical extract on work performance. In a series of acute experiments on normal non-adrenalectomized cats we have studied the actions of cortisone on muscular contractions. A soluble preparation (Hemisuccinate Merck) was used for intravenous and intra-arterial injections. Cortisone increased the amplitude and maintenance of muscular responses to electrical stimulation of the corresponding nerves. That increase was immediate and long lasting when single shocks were used. Similar effects were obtained when low frequency (20–40/sec.) tetanizing frequencies were employed. On the other hand, only small and transitory enhancements of the contractions were observed when higher frequencies were used. Cortisone improved the 5th stage of neuromuscular transmission when already developed, but prevented its appearance if the hormone was previously injected. Cortisone markedly increased the tolerance to ischemia of working striated muscle. We have tested cortisone in connection with a study of the actions of digitalis on muscle. The hormone produced a very marked potentiation of the effects of digitalis.

Mechanism of renal vascular shunt. E. C. DEL POZO, R. HERNANDEZ-PEON,* AND C. GUZMAN F.* *Inst. de Estudios Medicos y Biologicos, Univ. Nacional de Mexico, Mexico, D. F.*

Renal 'vascular shunt' produced by sympathetic stimulation as described by Trueta and collaborators has not been confirmed by most workers who have tried to reproduce it. The original Oxford group did not mention either the specific requirements for its appearance or the frequency of its production. We have tried to find out, by varying the intensity and duration of the stimuli applied to the nerves, if suitable conditions of the stimulus could be found for a constant or frequent development of the so-called by-pass. Cats under Dial anesthesia were used. India ink was injected for visualizing the renal vascular bed. We could not obtain the 'vascular shunt' with any of the wide range of intensities and durations employed. In a second series of experiments the ink injection was done at variable times (5–10 seconds) after the end of the stimulation. When this last procedure was followed, several kidneys showed an ink impregnation localized to the juxtamedular cortical zone coexistent with a peripheral ischemia. According to our results, we think that the 'vascular shunt' described by Trueta and collaborators is only a transitory phenomenon during the course of the vascular recovery from a total constriction produced by sympathetic activity.

Functional and histological studies of sympathetic ganglia infected with pseudorabies virus. JOHN DEMPSHER, FREDERIC B. BANG AND DAVID BODIAN (introduced by DETLEV W. BRONK). *Jenkins Labs. of Biophysics and Depts. of Medicine and Epidemiology, Johns Hopkins Univ. and Hosp., Baltimore, Md.*

Pseudorabies virus was injected into one eye of rats during ether anesthesia. Two to 4 days later the animals started vigorously scratching the face on the inoculated side. After onset of scratching both superior cervical ganglia were excised and action potentials were recorded from the preganglionic and postganglionic nerves. Impulses were found to be discharged spontaneously over the preganglionic and postganglionic nerves, always with ganglia from the inoculated side, never with ganglia from the other side or with ganglia from uninoculated animals. The spontaneous discharge was intermittent, bursts of activity occurring every few seconds. The discharge could be modified by preganglionic nerve stimulation, and was suppressed by certain chemical agents, in some cases by concentrations which did not block synaptic transmission. Amplitudes of action potentials elicited by preganglionic nerve stimulation were essentially normal in all ganglia. Histological examination showed that in ganglia from the innoculated side there were severe to complete chromatolysis of Nissl substance in essentially 100% of the cells, severe nuclear changes with inclusion bodies typical of pseudorabies infection, scattered pyknotic and partly autolyzed cells, and minimal inflamatory changes. Ganglia from the other side were histologically normal. Virus employed in these experiments was obtained from chorio-allantoic membranes of chick embryos infected with the Aujeszky strain of pseudorabies virus. Rats were inoculated with supernatant fluid from a 10% suspension of membrane in buffered saline.

In vivo and in vitro effect of ascorbic acid and ACTH on rat adrenal cortex. A. DESMARAIS AND J. LEBLANC (introduced by EDOUARD PAGÉ). *Inst. de Physiologie, Univ. Laval, Québec, and Defense Research Med. Lab., Toronto.*

Histochemical examination of adrenal glands of hypophysectomized rats given both ascorbic acid and ACTH showed a greater enlargement of the cortex and a greater decrease of sudanophilic substances, as compared to adrenals of hypophysectomized rats receiving ACTH alone. *In vitro* experiments on incubated slices of adrenal glands have shown that ascorbic acid and ACTH have a synergistic effect on the secretory activity of the cells of the adrenal cortex. (Supported by

a grant from the Defense Research Board of Canada.)

Taste sensitivity to homologous alcohols in oil. V. G. DETHIER. *Dept. of Biology, Jonhs Hopkins Univ., Baltimore, Md.*

Determinations of taste thresholds of alcohols in different solvents have been made according to the method of Harris and Kalmus (*Ann. Eugenics* 15: 24, 1949) in an attempt to elucidate the mechanism of action of these compounds on taste receptors. The median molar concentrations at which aqueous solutions of normal alcohols, from ethanol to octanol inclusive, can just be distinguished from water are: 0.32, 0.11, 0.028, 0.016, 0.002, 0.0013, 0.0006. These values agree satisfactorily with those of Renqvist (*Skandinav. Arch. f. Physiol.* 40: 117, 1920). When expressed as activities, the threshold values are approximately equal but exhibit a progressive increase from pentanol to octanol. Median molar concentrations at which mineral oil solutions of ethanol to octanol inclusive are just distinguished from oil alone are: 0.093, 0.043, 0.019, 0.013, 0.0098, 0.0092, 0.015. The ratio of oil threshold to water threshold increases as the series is ascended. In each case the ratio does not equal the corresponding oil/water partition coefficient determined interferometrically. These coefficients for ethanol to heptanol are: 0.06, 0.07, 0.11, 0.80, 16.0, 24.0. The relationship between the 2 series is linear. Inequality between corresponding values would suggest that during the process of stimulation the alcohol in the ambient solution is not in equilibrium with the solution at the site of action. However, since the tongue as a whole rather than a single receptor or taste bud was exposed to the stimulant, the disagreement may be an expression of factors other than non-equilibrium.

Electrocardiogram of the horse. D. K. DETWEILER (introduced by G. P. MCCOUCH). *Dept. of Physiology and Pharmacology, Univ. of Pennsylvania Veterinary School, Philadelphia.*

Electrocardiograms from 49 horses were analyzed. Of the 3 limb leads, *lead II* is usually the most favorable for analysis. QRS and QT values from this lead are usually almost identical with those from *lead III*, but somewhat longer than those from *lead I*. Mean values and their standard deviations from *lead II* are: P'P'', 0.059 ± 0.11; P, 0.151 ± 0.019; PR, 0.348 ± 0.078; QRS, 0.116 ± 0.025; QT, 0.532 ± 0.037 seconds; and systolic index (Bazett's formula) 0.402 ± 0.028. The mean heart rate was 35 ± 5/min. In 10 of the 49 horses partial heart block occurred. The TP:PR relationship characteristic of Wenckebach type of heart block in man is frequently reversed and the records suggest that momentary changes may occur in A-V node conductivity. The QT interval following the dropped beat is shortened, although in the cases studied the time interval between the first and second heart sounds is not shortened. Sometimes the T wave amplitude is lowered in the post-block complex. The P wave is frequently notched or slurred. Auricular electrograms indicate that the spread of excitation in the auricle is similar to that in the dog and that the cleft P wave represents asynchronous depolarization of the right and left auricular masses. Cyclic variations in the form of the P wave occur in some records. The QT/QRS and QT/$\sqrt{RR}$ ratios are similar for various species (elephant, horse, ox, man, dog) with widely differing cardiac rates and heart size.

Phospholipide metabolism in phlorizinized dogs. N. R. DILUZIO* AND D. B. ZILVERSMIT. *Div. of Physiology, Univ. of Tennessee, Memphis.*

The metabolism of liver and plasma phospholipides was studied with radioactive phosphorus in normal and fasting phlorizinized dogs. Chemical and radioactive determinations were made on liver and plasma phospholipides and on the following phosphate fractions: plasma inorganic-, liver inorganic-, and liver organic acid soluble-phosphate. The turnover of liver and plasma phospholipides was determined by specific activity and relative specific activity ratios. In comparison to the control group, the following alterations were noted in the phlorizinized dogs. There was a four-fold increase in the specific activity ratios of liver and plasma phospholipides, but there was no change in the amount of liver phospholipides per gm. of fresh tissue. The level of plasma lipides was elevated and excessive deposition of fat was noted in the liver. The amount of liver organic acid soluble phosphate fraction per gm. of fresh tissue was significantly reduced. Plasma and liver inorganic phosphate were nearly equal to the control values. This study, as well as that previously reported on the depancreatized dog (*Federation Proc.* 10: 150, 1951) shows that when fat catabolism is greatly exaggerated, the turnover of liver phospholipides is markedly increased. Although the primary function of liver phospholipides may be of a nonmetabolic nature, our findings suggest that some phospholipides may play an active role in fat oxidation. (Supported by a grant from the American Cancer Society.)

Effect of temperature of heart on susceptibility to ventricular fibrillation. JOSEPH R. DIPALMA. *Hahnemann Med. College, Philadelphia, Pa.*

It was found possible to produce a permanent type of ventricular fibrillation in the cat by the application of constant DC currents of from 1–5 ma. for 5 sec. Non-polarizing electrodes were used. The positive electrode was attached to the surface of the left ventricle. The temperature of the heart

was recorded by a fine thermocouple placed directly beneath the heart. Alterations in rhythm were followed by direct observation and continuous recording ECG. It was noted in the warm animal that pouring of cold saline (20°C.) on the heart made restoration of sinus rhythm impossible. On the other hand pouring warm saline (45°C.) on the heart in most instances restored normal rhythm without other procedures. After control observations were made the cat was placed in a refrigerating unit and the temperature of the heart was brought down gradually from the normal of 38–39° to as low as 15° in some experiments. The threshold as measured by the amount of current needed to produce fibrillation did not change consistently. On the other hand, in the cold the tendency for heart block to occur on passage of the current through the auricles instead of the ventricles was greatly increased. Ventricular fibrillation occurred more frequently and with smaller currents in the presence of heart block.

Colloid disappearance rate as a measure of liver blood flow. ERNEST L. DOBSON,* GEORGE F. WARNER* AND HARDIN B. JONES. *Donner Lab., Univ. of California, Berkeley.*

When colloidal chromic phosphate (DOBSON AND JONES. *Acta Med. Scandinav.*, in press) is injected into the blood stream it disappears at an exponential rate. The disappearance is due to phagocytosis primarily by the macrophages of the liver and spleen. Blood clearance by these phagocytes is nearly complete. The concentration of the colloid in the blood at any time can be expressed by the general equation,

$$C = C_1e^{-k_1t} + C_2e^{-k_2t} + C_3e^{-k_3t}$$

The fastest component of this multi-component exponential curve is a function of the liver blood flow. Liver perfusion measurements obtained in a number of mammalian species vary somewhat but have been found to be in the neighborhood of 1 volume of blood for each volume of liver tissue/minute. The only non-mammalian species studied is the chicken (RANNEY, CHAIKOFF AND DOBSON. *Am. J. Physiol.* 165: 588, 1951) which has a very much higher liver perfusion rate, about 3½ volumes of blood/volume of liver tissue/minute. The colloid disappearance rate constant has a value of 0.28 min.$^{-1}$ in young normal men (DOBSON, WARNER, RODGERS AND JOHNSTON, to be published). This corresponds to a blood tissue perfusion factor of slightly more than 1 volume of blood/volume of liver tissue/minute. Postprandially the liver circulation shows an increase while the response to epinephrine has been found to vary considerably from individual to individual. The application of this method to the study of patients with liver disease is in progress.

Hormone excretion in diffuse liver disease. C. DOHAN, E. RICHARDSON,* L. BLUEMLE, JR.* AND P. GYORGY.* *Dept. of Medicine and Nutritional Service, Univ. of Pennsylvania, Philadelphia.*

The urinary excretion of the unconjugated and conjugated forms of the 'estrone,' 'estradiol' and 'estriol' fractions, as well as neutral 17-ketosteroids and gonadotrophins, was determined for one or more 3–22 day periods in 5 'normal' men and 17 men with diffuse hepatic disease. The results support the concept of disturbed hormone metabolism as a cause for some of the symptoms of diffuse hepatic disease and suggest that decreased biliary excretion of estrogens may, in part, be responsible for increased urinary excretion. The results are listed below: *1*) Total estrogen excretion was increased in 11, and the unconjugated form in 6 patients. *2*) Excretion of total estrogens was significantly correlated with bromsulphalein retention and total serum bilirubin values in patients with mild jaundice. *3*) Excretion of total estrogens was significantly increased in the 4 patients with advancing or stable gynecomastia in comparison to that of patients with regressing or no gynecomastia. *4*) Both forms of estriol were increased to a greater extent and more frequently than estrone or estradiol. *5*) Excretion of unconjugated estriol was significantly increased in patients exhibiting stable or advancing cutaneous spider nevi. *6*) The 17-ketosteroid excretion was less than normal in 14 patients and was decreased in the 4 men with stable or advancing gynecomastia and the 6 men with testicular atrophy and/or complete loss of libido when compared to patients without these symptoms. *7*) Gonadotrophins were significantly decreased in the 6 patients with testicular atrophy and/or complete loss of libido.

Protection of α-chymotrypsin against gamma radiation. DAVID G. DOHERTY (introduced by ALEXANDER HOLLAENDER). *Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.*

Electrophoretically homogeneous α-chymotrypsin was prepared from bovine pancreas by the usual procedures, lyophyllized and utilized for all the radiation experiments. Enzymatic activity was determined by following the rate of hydrolysis of glycyl-L-tyrosine amide with a potentiometric formol titration. Dilute chymotrypsin solutions were exposed to γ-ray dosages from 1000 to 120,000 r from a cobalt source at the rate of 140 r/min.; maximum inactivation of 30% was obtained at the 40,000-r level. Dry chymotrypsin irradiated at dosages from 500,000 to 10,000,000 r gave essentially similar results although shifted to a higher dosage level. The Km remained unchanged from all the irradiated enzyme indicating that there had been no alteration in the active centers of the enzyme

molecule. This was substantiated by binding studies with a radioactive tagged substrate by the procedure of Doherty and Vaslow (*J. Am. Chem. Soc.* Jan. 1952). Since substrates protect enzymes against radiation damage it might be expected that competitive inhibitors would show the same effect. Adjustment of concentrations might yield conditions which would give protection but very little inhibition. Thus chymotrypsin was irradiated with 20,000 r in the presence of several inhibitors, i.e. acetyl-L-tyrosine, 4-*p*-hydroxyphenyl-3-acetamino-butanone-2, *p*-hydroxyphenyllactic acid and phenyllactic acid. Inhibition varied from 2 to 50% while, with the exception of phenyllactic acid—which gave 50% protection, the other inhibitors afforded complete protection to the enzyme. Different inhibitors are being investigated in an effort to gain some insight into the structural part of the enzyme damaged by radiation.

Intercostal nerve electrical activity. A. S. Dontas* and Robert Gesell. *Physiology Lab., Univ. of Michigan, Ann Arbor.*

The activity of intercostal ventral roots of dogs and cats was studied electrically along with mechanical recording of breathing. The electrical records of eupnea showed either no activity, periodic discharge of inspiratory or expiratory fibers or of both, and sometimes continuous activity at lower levels. Dogs, and in lesser degree cats, show normally active inspiratory neurons predominate, in general, above T8 and expiratory neurons below this level. Periodic bursts of impulses, inspiratory and expiratory, are superimposed upon slow negative electrotonic waves (d.c. amplifier) with expiratory spikes showing lesser adaptation to slowly rising currents. Chemoceptor stimulation (NaCN) elicits discharges from dormant neurons of both kinds, combined with a long-lasting elevation of the mean level of ventral root potential. Selective excitation of antagonistic neurons was obtained by clamping of the trachea timed to obstruct either inspiration or expiration, the latter, e.g. resulting in prolonged and increased expiratory discharges, based on greatly enhanced electrotonic waves. Short stimulations of dual excitatory nerves (sup. laryngeal) tended to prolong selectively the discharges of the motoneurons active at the moment of stimulation, and to inhibit their antagonists. This inhibition is thought to result from the overactivity of one set of neurons reciprocally depressing the antagonists. Inhibition of motoneuron activity, inspiration and expiration, obtained with Na_2CO_3 infusions was associated with long-lasting depression of the mean level of the ventral root potential.

Anterior pituitary gland, growth hormone and secretin content of rat intestine. J. E. C. Dorchester* and R. E. Haist. *Dept. of Physiology, Univ. of Toronto, Toronto, Canada.*

The secretin content of extracts of the entire small intestine in rats was determined. The finding that hypophysectomy reduced the total secretin content of the intestine and also reduced the concentration of secretin per gram of dried intestine (*Federation Proc.* 10: 36, 1951) has been extended. Injections of a crude saline extract of the anterior pituitary gland prevented this reduction in secretin levels in hypophysectomized animals. Injections of this crude extract into intact rats in most instances caused an increase in the total content and concentration of secretin above the normal levels. Administration of desiccated thyroid gland did not prevent the reduction in secretin levels in hypophysectomized rats. Hence the pituitary effect probably is not mediated through the thyroid gland. In a small series, the daily injection of a highly purified growth hormone preparation (Frank W. Horner Ltd.) was effective in preventing the reduction in secretin levels in hypophysectomized animals.

Comparison of three methods for repeated rapid determinations of cardiac output. Philip Dow, John W. Remington and Albert R. Howard.* *Dept. of Physiology, Med. College of Georgia, Augusta.*

Most of the currently respected methods for estimating cardiac output are either too slow to follow transitory changes or too laborious for frequent repetition in a single experiment. This study reports comparisons of some possible improvements. The Nahas cuvette for rapid spectrophotometric oximetry of whole blood has facilitated the direct Fick determinations but leaves this method limited by the adequacy of the oxygen consumption measurement. The logarithmic photoelectric densitometer developed by E. V. Newman (after Morgan, from original circuit by Sweet) and the polyethylene carotid loop cuvette for it (Pearce, this program) have overcome many of the limitations of the dye injection method for repeated estimates. Practically simultaneous determinations by these two methods (hitherto untested for extreme range of rapidity and repetition) have been made on anesthetized dogs under measures which produced various combinations of changes in pressure and output. Comparability was controlled by reading aortic pressure contours recorded during the actual time span of each method. Small amounts of T-1824 do not interfere with the spectrophotometric oximetry, but repeated injections require the precaution of an extra reading at 625 mμ to detect such interference and correct for it if present. Results of this three-way comparison will be presented, with recommendations for the range of usefulness of each.

An evaluation of dye-dilution method of measuring cardiac output and so-called pulmonary blood volume. JOSEPH T. DOYLE,* JOSEPH S. WILSON,* CHARLES LEPINE* AND JAMES V. WARREN. *Emory Univ., Atlanta, Ga.*

By injecting T-1824 into a cardiac catheter and collecting arterial blood during first circulation of the dye, one can measure mean circulation time (MCT), cardiac output (CO) and so-called pulmonary blood volume (PBV). Approximately 300 nearly simultaneous measurements in human subjects of blood flow by the dye-dilution and Fick methods permit the following conclusions: CO measured by the dye and Fick methods correlates satisfactorily. The dye method may be unreliable if CO is extremely low, and is invalid in the presence of intracardiac shunts and free aortic regurgitation. The dye method is adequately reproducible. The measurement of PBV, on the other hand, is poorly reproducible. PBV is not correlated with pulmonary arterial or capillary pressure, pulmonary vascular resistance or vital capacity. A correlation with stroke volume is inherent in the method. Artificial expansion or reduction of general blood volume (GBV) does not alter the ratio PBV:GBV. In hypokinetic heart failure PBV is large, while in hyperkinetic failure PBV is essentially normal. In both types of failure PBV:GBV is maintained at the normal 25%. Mitral stenosis (MCT prolonged; PBV and PBV:GBV large) and tricuspid insufficiency (MCT slightly prolonged; PBV and PBV:GBV relatively small) alone show significant contrast. Tilting and venous pooling of blood produce a statistically equivocal reduction in measured PBV. It is concluded that the dye measurement of CO is useful while the measurement of PBV is relatively insensitive in quantitating small but physiologically important changes in volume.

Alveolar O_2 and CO_2 during inspiration, expiration and breath holding. A. B. DUBOIS (introduced by W. O. FENN). *Dept. of Physiology and Vital Economics, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Data are presented showing changes in alveolar gases during breath holding. During the first 20 seconds the P_{O_2} diminishes linearly with time according to an O_2 equation. An equation is developed for the changes in P_{CO_2} which shows that the P_{CO_2} approaches the oxygenated venous P_{CO_2} exponentially so long as the venous blood remains unchanged. The exponential constant of the CO_2 equation is $-\frac{\dot{Q}bS(B-47)\,t}{V_A - C_{LCO_2}}$ where $\dot{Q}b$ is the blood flow, S is the slope of the oxygenated CO_2 dissociation curve, V_A is the alveolar volume and C_{LCO_2} is the capacity of the lung tissue and lung capillary blood for CO_2. Combining the O_2 and CO_2 equation with the elimination of t gives another exponential equation which describes the breath holding pathway on the P_{O_2}-P_{CO_2} diagram. The initial slope of this curve is equal to $\bar{R}\,V_A/(V_A + C_{LCO_2})$ and in 10 subjects had an average value of $\bar{R} \times 0.83 \pm$ S. E. 0.08, where $\bar{R}$ is the mean respiratory gas exchange ratio. This provides therefore a method of evaluating C_{LCO_2}. This equation also gives the changes in alveolar concentration which occur during expiration which is comparable to breath holding. An equation for the alveolar changes during a large inspiration has also been worked out and serves as a pattern for the alveolar changes which occur during inspiration. In normal breathing expiration and inspiration form a counter clockwise loop around the mean alveolar point on a P_{O_2}-P_{CO_2} diagram with a 2 mm. change in P_{O_2} and a 3 mm. change in P_{CO_2}.

Radiosensitivity of *C. paramecium* as influenced by phase of nitrogen assimilation. HOWARD S. DUCOFF (introduced by D. E. SMITH). *Div. of Biological and Med. Research, Argonne Natl. Lab., Chicago, Ill.*

The duration of x-ray-induced division block in *C. paramecium* has been previously reported to be the same, whether cells were in logarithmic phase of growth or were nonproliferative due to nitrogen depletion. Nitrogen-depleted cells to which a nitrogen source is restored just prior to irradiation are much more radiosensitive than cells in any other phase of nitrogen assimilation. This increased sensitivity is manifested in either or both of two ways: a greater degree of killing at higher doses (30,000 r) or a second division block following apparent recovery from a division block of normal duration at lower doses (15,000 r). Similar experiments using nitrogen mustard (*HN-2*) also indicate that the time course of cell division is a function of the time since restoration of the nitrogen source. Cells given nitrogen supply shortly before exposure appear to be more sensitive to mustard action than those given nitrogen either longer before or simultaneously with the mustard. These apparent differences between nitrogen mustard and x-ray in regard to pattern of nitrogen assimilation and sensitivity may be real, or else may be due to differences in the time-intensity pattern of exposure to the two agents.

Ascorbic acid and acclimatization to cold in monkeys. LOUIS-PAUL DUGAL AND GUY FORTIER.* *Inst. de Physiologie, Faculté de Médecine, Univ. Laval, Quebec.*

Acclimatization to cold was appreciated by resistance to intense cold (2 hours at −20°C.),

as reflected by the drop in rectal and intramuscular temperature. Eighteen monkeys (*Rhesus maccacus*) were divided in 3 groups: the first group was kept at room temperature and fed Purina Dog Chow plus a supplement of 25 mg/day of ascorbic acid; the second group, fed the same diet as *group 1*, was pre-exposed to a temperature of + 10°C. for 6 months before being tested for resistance to −20°C.; the third group was treated like the second one, but received in addition 300 mg/day of ascorbic acid. The results obtained in more than 80 experiments show that monkeys pre-exposed to mild cold during 6 months and receiving 25 mg/day of vitamin C (which is an important amount when one considers the weight of those animals) do not resist intense cold better than controls kept at room temperature. That result is contrary to what has been found in animals able to synthesize ascorbic acid, e.g., rats. On the contrary, monkeys pre-exposed to cold for the same period and receiving 325 mg. daily of ascorbic acid (300 mg. more a day) are far better resistant *a*) than the controls kept at room temperature and *b*) than the controls also pre-exposed to cold but receiving only 25 mg. daily of ascorbic acid, when exposed (at rest) for 2 hours at −20°C. All those results are statistically highly significant. (Supported by a grant from the Defense Research Board of Canada.)

Effects of ascorbic acid and ACTH combined or separately on adrenal weight of normal and hypophysectomized rats. Louis-Paul Dugal and Mercedès Thérien.* *Inst. de Physiologie, Univ. Laval, Quebec.*

From observations made in our laboratory and others, ascorbic acid has been reported to increase the fall in adrenal cholesterol during exposure to cold, and to prevent, at the same time, the normal hypertrophy of the adrenals. Resistance to cold is nevertheless increased by ascorbic acid. The purpose of the present investigation was to try to clarify and connect the above findings. In normal rats, ascorbic acid does not prevent the action of injected ACTH on the adrenal. In hypophysectomised animals, ascorbic acid alone does not replace the action of ACTH, but the same substance does potentiate the effect of ACTH on adrenal weight. In other words, small doses of ACTH which have no effect by themselves on the adrenal weight of hypophysectomised rats, have the same effect as large doses of the same substance, if they are administered together with ascorbic acid. (Supported by a grant from the Defense Research Board of Canada.)

Methods of fractionating Pitressin. Mary E. Dumm, Stephen H. Leslie* and Bertram Laken.* *Dept. of Medicine, College of Medicine, New York Univ.-Bellevue Med. Center, New York City.*

When Pitressin was treated with 0.01 M sodium thioglycollate and assayed in rats, most of the antidiuretic activity was retained but the chloruretic and pressor activities were destroyed (Ralli *et al. Am. J. Physiol.* 163: 141, 1950). In the experiments reported in the present study active antidiuretic preparations have been obtained after adsorption on either alumina gel or oxycellulose. Cation exchange resins were not satisfactory adsorbents for Pitressin. The method used was to absorb Pitressin (200 mm/ml.) on alumina gel at a pH of 5.1, which removed about 50% of the antidiuretic activity of the solution as measured by the rat assay. The alumina gel was eluted with 0.05 M phosphate buffer at a pH of 7.8 and the eluate again assayed in rats. From 10–20% of the original antidiuretic activity was recovered with no evidence of any associated chloruretic or pressor activity. In the experiments with oxycellulose Pitressin (50 mm/ml.) in 0.1 M acetic acid was stirred with oxycellulose (10.9% COOH) for 5 hours which removed all but traces of antidiuretic and chloruretic activity from the solution. The oxycellulose was eluted with 0.1 M HCl and an aliquot neutralized, diluted and assayed in rats. Somewhat more than 100% of the antidiuretic and chloruretic activity of the original solution was recovered at more than 5 times its original concentration.

Tissue electrolyte and water changes after glucose per os in epinephrine protected and unprotected adrenalectomized rats. Abraham Dury and Thomas N. Johnston.* *Dorn Lab. Med. Research, Bradford Hosp., Bradford, Pa.*

Within 20 minutes after 2 ml. 50% glucose solution was admitted into the stomach of 250 gm. adrenalectomized rats, 80% of the group showed signs of mortal failure. When adrenalectomized rats were pretreated with epinephrine 60 minutes before the glucose solution was administered, 80% of the group survived without signs of failure. The changes in the water and electrolyte concentration of plasma, skeletal muscle and liver after glucose administration in the non-surviving group not pretreated with epinephrine and the surviving epinephrine-pretreated group were compared with their respective controls. Evidence will be presented that mortal failure in the unprotected group resulted from the rapid increase in plasma K concentration and the cardiac changes associated with hyperkalemia. A large significant decrease in the potassium concentration of the liver was found in this group but no changes were found in the plasma sodium, chloride of water nor the concentration of water or electrolytes in the skeletal muscle. Epinephrine pretreatment induced a significant decrease in plasma and muscle

K concentration. This apparently was an important factor in protecting this group from failure after glucose administration since no changes were found after more than 20 minutes survival in the electrolyte concentration of plasma, muscle or liver. The results will be discussed as indicating a significant role of epinephrine in potassium homeostasis.

Glycemic levels and rumen development in calves. J. A. DYE AND D. ORSINI.* *Dept. of Physiology, Cornell Univ., Ithaca, N. Y.*

An inverse relationship between the glycemic level and functional development of the rumen in domesticated and wild ruminants has been reported from this laboratory (*Am. J. Physiol.* 162: 434, 1950). Further studies on 2 male calves have shown an influence of the diet on this relationship. Postabsorptive glycemias of newborn calves range from 90–125 mg/100 ml., but decrease rather rapidly during the first postnatal month, then more slowly until adult levels of 40–60 mg/100 ml. are reached. This decline is appreciably slower when the diet consists of milk alone than when the animals have, in addition, free access to calf starter and hay. A shift from the latter to the former diet is accompanied by an appreciable glycemic rise and vice versa. Postprandial hyperglycemic curves subsequent to drinking 6 quarts of milk were higher during periods of milk feeding than during those in which the milk was supplemented with hay and grain—expecially when the milk was taken from a nursing pail. An apparent explanation of these observations concerns the rapidity with which the feed reaches the abomasum and inversely with the degree of bacterial action on the feed before it reaches this compartment of the stomach. In 7 experiments on a 6.5-month-old calf, with an abomasal fistula, receiving milk, hay and grain, the abomasal content was found to be completely free of yeast-fermentable sugar. However, after acid hydrolysis, at pH 1, appreciable quantities of fermentable and non-fermentable reducing substances were present. Continued studies are in progress.

Effect of ACTH on renal function of hypophysectomized dogs. D. P. EARLE, S. J. FARBER,* R. C. DE BODO AND M. KURTZ.* *Depts. of Pharmacology and Medicine, New York Univ. College of Medicine, New York City.*

Glomerular filtration rate (GF) and renal plasma flow (RPF) are greatly reduced in hypophysectomized dogs. Five such dogs were placed on an ACTH regimen (DE BODO *et al.*, *Federation Proc.*, this issue) sufficient to produce regeneration of the atrophic adrenals and to abolish insulin hypersensitivity. GF and RPF increased in each dog, the mean postoperative value for GF increasing from 13 to a peak value of 42 ml/min. and RPF from 63 to 151 ml/min. The increase was apparent at the earliest observation, viz. on the 8th day of the ACTH regimen; it was progressive, but in no instance did renal functions return to preoperative control values. Renal functions were routinely measured 2–3 hours after the morning dose of ACTH, but in 2 dogs observations were also made when the morning dose had been omitted. In 3 of the 4 such observations on these dogs, GF and RPF were as low as the pre-ACTH values, although these dogs had been on ACTH for 21 and 115 days prior to these studies. Three observations on the acute effect of ACTH in these 2 dogs indicated that GF and RPF did not increase until 30 to 90 minutes after the administration of ACTH and reached their peak values in approximately 2 hours. The ACTH effect on renal function appeared to be greater than that of growth hormone, when both were given in doses that abolished the insulin hypersensitivity of hypophysectomized dogs.

Facilitation in crustacean neuromuscular system. DEXTER M. EASTON (introduced by T. C. RUCH). *Zoology Dept., Univ. of Washington, Seattle.*

The extensor muscle of the dactyl of a crustacean is supplied by two fibers that run parallel to one another to the terminals, where they end in numerous branches on each muscle fiber. The larger of these fibers is excitatory, while the smaller inhibits the responses obtainable from stimulation of the larger fiber. Each impulse delivered to the terminals of the excitatory nerve produces a neuromuscular synaptic potential, similar to the end-plate potential of vertebrate skeletal muscle. The amplitude of each potential depends upon the previous history of excitation of the nerve. In a rested preparation, initial potential height may be zero during a train of pulses, but successive potentials will increase in height at a rate depending upon the frequency of stimulation. This facilitation occurs at frequencies of stimulation as low as 10/sec. There is appreciable post-tetanic potentiation of both the contraction and of the neuromuscular potentials. After a 5-sec. tetanus of the excitatory nerve at 100/sec., the neuromuscular synaptic potential recorded within 5 sec. may be 300% of normal, and require several minutes to return to normal. The facilitation and potentiation process are explained in terms of negative after-potential of the nerve which may last for 100 msec. after a single impulse, and for several minutes after a short tetanus. The small fiber size and extensive branching probably produce a low safety factor of conduction. During the negative after-potential, conduction into these terminals may be facilitated, and a larger area of muscle brought under their influence.

The aorta to coronary sinus anastomosis of Beck. RICHARD W. ECKSTEIN, CARL HORNBERGER,* GEORGE SMITH* AND DAVID LEIGHNINGER.* *Dept. of Medicine, Western Reserve Univ., Cleveland, Ohio.*

Beck has shown in dogs that his operation reduces the mortality and size and number of infarcts following coronary artery ligations. We have also shown in acute open chest dogs that the addition of arterial blood to the cannulated coronary sinus, at a mean pressure of 50 mm. Hg, reduces the mortality during the first hour following circumflex artery ligation. These present studies deal with the mechanism of benefit *1)* in acute experiments on normal dogs, and *2)* in acute experiments on dogs previously prepared by the Beck technique. Information has been obtained concerning the changes in peripheral coronary pressure, amounts of peripheral coronary back flow and its oxygen content. Electrocardiographic records have been made. Present evidence indicates the reduction in mortality is due to a few cc. of blood which pass in a reversed direction through capillaries from the coronary sinus to the occluded artery. Such blood gives up its oxygen. The reversed flow decreases in a matter of hours in acute dogs following major artery ligation and persists for some days in chronic dogs, after which time the usual interarterial collaterals enlarge. However, when the anastomosis has been in place for 1 month in the presence of open coronary arteries, potential channels for reversed flow can be shown to remain open. Ischemic electrocardiographic changes are induced by arterializing the coronary sinus when coronary flow is reduced. Severe ischemic changes resulting from complete artery occlusion are slightly improved by sinus arterialization.

Contractile system of cardiac muscle. NANCY CANTOR EDDY* AND F. J. M. SICHEL. *Dept. of Physiology and Biophysics, Univ. of Vermont College of Medicine, Burlington.*

One of us has previously shown that in isolated skeletal muscle fibers with cut ends, the contractile mechanism can be excited to a normal type of response apparently without involving the conducting mechanism. The intact entire sartorius can respond similarly provided the KCl content of the medium is raised sufficiently high to abolish conduction. The present experiments show that cardiac muscle (ventricular and atrial strips from the turtle heart) can react similarly. As in the previous experiments, since the muscle is rendered non-conducting, the mechanical response is restricted to the region stimulated. To obtain a response of reasonable size, the entire length of the muscle strip was stimulated simultaneously through massive electrodes placed so the current would flow uniformly across the muscle strip. The stimuli were rectangular current pulses of variable strength and duration. The contractions were recorded isometrically. As in voluntary muscle the response of the contractile system is gradable, the maximum isometric tension and the duration of the contraction depending on both the strength and duration of the shock. A stimulus of duration comparable to the normal depolarization time produces a contractile response of about the same duration and form as the normally propagated contraction. A very brief stimulus elicits a twitch-like response occurring entirely after the stimulus. There is apparently a threshold for the response, but no refractory period. (This investigation was supported by a research grant from the National Heart Institute, of the National Institutes of Health, U. S. Public Health Service.)

Centrifugation of animals about an axis through the body. R. EDELBERG AND H. S. WEISS (introduced by H. M. SWEENEY). *Aero Med. Lab., Wright Air Development Center, Wright-Patterson Air Force Base, Dayton, Ohio.*

The rapid tumbling that often accompanies emergency ejection from aircraft represents an unknown threat to the flyer's safety. In experiments designed to simulate this tumbling, dogs, anesthetized with Nembutal and lying on either side, were spun on a horizontal turn-table with the heart at the center of rotation. Respiratory rate, EKG and direct measurement of blood pressure from 4 vessels were recorded simultaneously. The centrifugal force induced by the spin about the heart opposes venous return, resulting, at speeds above 120 rpm, in a picture of acute hypoxia. Cardiac output is decreased, as indicated by the marked reduction in arterial pressure, pulse pressure and A-V pressure difference. Tachycardia develops and is primarily attributal to the abrupt, acute fall in carotid pressure. Breathing ceases in the inspiratory phase probably as a result of a caudad displacement of the diaphragm. Animals survived exposures to 200 rpm for 2 minutes but developed subendocardial and cerebral subarachnoid hemorrhages. Similar subendocardial hemorrhages have been produced by the beating of an 'empty' heart. The subarachnoid ecchymoses are attributed to the sudden rise in systolic pressure from zero to almost 450 mm. Hg immediately after the run. The effect of anoxia on the strength of these vessels may be a factor. Measured pressures, after cardiac output had fallen to near zero, were lower than calculated hydrostatic values, suggesting that vascular pressures cannot be predicted on the basis of a simple rotating column of fluid.

Penetration of sodium and water into bone as measured with radiosodium and D_2O. I. S. EDELMAN, A. H. JAMES AND F. D. MOORE (in-

troduced by J. R. PAPPENHEIMER). *Peter Bent Brigham Hosp. and Harvard Med. School, Boston, Mass.*

Studies on the penetration of radiosodium and D_2O into bone were carried out as a preliminary to determining the availability of these stores for use in metabolic emergencies. Inasmuch as these stores constitute approximately 35% of body sodium and 10% of body water their role in metabolic processes may be of considerable significance. In 7 dogs and 1 human, blood and bone biopsies were obtained over a 50 hour period after intravenous administration of isotonic $Na^{24}Cl$. After distribution equilibrium, the ratio of the specific activity in bone to that in serum is a measure of the fraction of the element exchanged. Biopsies of rib, skull and femur showed that 40–55% of bone sodium exchanges in the first 3–24 hours. Further studies were carried out in 2 dogs injected with Na^{22} ($T_{\frac{1}{2}}$ = 3.0 yrs.) and maintained on rice diets. Serial bone biopsies over a 4 week period did not yield any further exchange. These data demonstrate that a large fraction (approximately 55%) of bone sodium is bound in matrix. Parallel studies with D_2O were carried out in 6 dogs. For a post equilibration interval up to 30 hours, a maximum of 80% water exchange was obtained for rib and femur cortex. The implications of these results for the interpretation of water and electrolyte distribution based on dilution studies will be discussed.

Effect of nitrogen mustard on water metabolism in rat. ABRAHAM EDELMANN, RUTH B. HEALEY* AND GLADYS M. MATEYKO.* *Biology Dept., Brookhaven Natl. Lab., Upton, N. Y.*

Nitrogen mustard was injected intravenously in doses of 0.025, 0.05, 0.1 and 0.2 mg., into male albino (Sprague-Dawley) rats of about 220 gm. weight. After 0.05 or 0.1 mg. there was a persistent diuresis. The urine volume was quite large for the first 24 hours after which time it fell gradually for 12 days to pretreatment values. Following 0.2 mg., urine volume increased slightly for 2–3 days and slowly returned to normal by 10–12 days. Only a slight increase in urine volume at 3 days was evident after 0.025 mg. Water intake varied as the urine output. A test dose of water was given by stomach tube 2 hours after injection of 0.1 mg. Control animals were similarly treated except they were injected with normal saline instead of the mustard. The experimental animals excreted the water much more rapidly than the controls. Serum taken at various times, up to 24 hours, from N-mustard treated rats was assayed for antidiuretic activity. There was no apparent change in the blood titer of the antidiuretic substance. After whole body x-irradiation these phenomena occur but are accompanied by a decrease in circulating antidiuretic substance. It would appear, therefore, that while the diuresis and response to administered water are similar to those seen after whole body x-irradiation, the mechanisms involved are different. (Research carried out at Brookhaven National Laboratory under the auspices of the Atomic Energy Commission.)

Temperature regulation in conscious dog during thermal stress. H. E. EDERSTROM AND A. C. HIGGINBOTHAM.* *Dept. of Physiology, St. Louis Univ. Med. School, St. Louis, Mo.*

The temperature of several skin areas in conscious dogs rose about 1°C. when the animals were transferred from an environmental temperature of 25°C. to one of 36°C. During the next 3 hours skin temperature remained relatively constant. In the same experiment, rectal, tongue, and air temperature in the buccal cavity did not change appreciably. The decrease in the temperature gradient between skin and room air was offset mostly by increased heat loss from the respiratory tract, resulting in thermal equilibrium. Arterial perfusion of the footpad and ear, measured with a photoelectric plethysmograph, showed little change during the experiment. Cutaneous evaporative losses, measured by a desiccating capsule, increased in the hot environment. The rise in evaporation was considered the result of increased secretion by the sebaceous glands. The iodine starch-paper technique yielded spots from the dog skin in cold as well as hot environments. No evidence of thermal sweating comparable to that in the human skin was noted in the dog. At air temperatures of 36°C. cutaneous evaporation accounted for about one-fifth of the total heat loss of the dog, the remainder apparently being dissipated through the respiratory route. (Aided by a grant from the U.S. Public Health Service.)

Consumption of glucose by sympathetic ganglia. CHARLES EDWARDS* AND MARTIN G. LARRABEE. *Jenkins Labs. of Biophysics, Johns Hopkins Univ. and Hosp., Baltimore, Md.*

Consumption of glucose by superior cervical ganglia excised from rabbits was measured at 35°–37° C. The ganglion (weight approximately 8 mg.) was mounted in a small plastic chamber with its preganglionic and postganglionic nerves in contact with stimulating and recording electrodes. Bathing solution containing sodium, potassium, calcium, magnesium, chloride, phosphate, and glucose (1 gm/l.) was equilibrated with 5% CO_2, 95% O_2 and buffered with bicarbonate to pH 7.4. This solution flowed through the chamber except during periods of glucose uptake determination. For each determination the ganglion was bathed in 0.02 ml. of solution, which was raised and lowered by a piston, thereby alternately exposing the ganglion to the gaseous phase and to the solu-

ion. After 15–45 minutes, solution was withdrawn and analyzed for glucose. This was done by drying the sample, redissolving in 0.05 ml. of oxygenated solution containing glucose oxidase (notatin), and finally measuring, with a polarized platinum electrode, the oxygen concentration remaining after oxidation of the glucose. The average glucose consumption at rest was 1.25 μg/mg/hr. (extremes: 0.79 and 2.11 μg/mg/hr.). Repetitive stimulation at 4/second and 10/second did not, on the average, significantly accelerate the uptake of glucose, in contrast to the considerable acceleration of oxygen uptake previously observed (e.g. 20% at 4/second and 40% at 10/second). The glucose uptake was accelerated 50–100% by removal of oxygen or addition of NaCN, and was markedly decreased on lowering the temperature to 25°.

Survival rates in experimental burn shock. ALBERT EINHEBER,* LOUIS P. MUNAN* AND CHESTER E. LEESE. *Dept. of Physiology, George Washington Univ. School of Medicine, Washington, D.C.*

In the course of investigations in the chemoprophylaxis of burn shock, several untreated groups representing a total of 300 animals were used to demonstrate the effect of burns on survival rates in rats subjected to variations in the temperature of the burn (65, 70, 73, 75, and 80°C.) and in duration of the burn (10, 15, and 20 sec.). A standard burn was obtained in animals anesthetized with sodium pentobarbital 15 minutes prior to immersion of approximately 90% of the body surface area in water maintained at constant temperatures. In burns of 75°C. and above, the highest mortality occurred within the 12-hour period following the burn. All animals burned at 70°C. and above eventually died of burn sequelae. These data support the work of S. J. Hazan and C. R. Treadwell (*Proc. Soc. Exper. Biol. & Med.*, 68: 684, 1948). Most of the survivors of the 65°C. burn developed a transient, well-circumscribed alopecia in unburned areas of the head. Of interest too is the consistent observation that in the immediate period following burn the males regained consciousness and showed signs of activity earlier than the females. The color attending the edema of the burned limbs seemed to vary with the temperature of the burn, lower temperature ranges produced a shade of red, which merged into purple at higher temperatures.

Effect of oxypolygelatin, plasma and 0.9% sodium chloride solution on plasma volume of human subjects after hemorrhage. JOHN D. ELDER, JR., HAROLD G. BARKER AND JAMES M. WALKER (introduced by ROBERT D. DRIPPS). *Harrison Dept. of Surgical Research and Dept. of Anesthesiology, Schools of Medicine, Univ. of Pennsylvania, Philadelphia.*

Recent clinical experience has established the usefulness of several plasma expanders in the treatment of hypotension; however, their relative value is difficult to judge from these observations alone. It was believed that they could better be compared on the basis of their ability to increase and maintain plasma and blood volumes. A series of young males in good health were subjected to a controlled hemorrhage of approximately 1000 cc. followed immediately by an infusion of an equal volume of the plasma expander being tested. Heparinized human plasma, oxypolygelatin (Baxter S152AY), and physiological saline have been studied to date. Plasma volume measurements were made using T-1824 before infusion, at 4 hours, and 12 hours after infusion. Hemoglobin, hematocrit, and plasma protein were followed for 24 hours, and, in the subjects receiving oxypolygelatin, analyses have been carried out for this substance in the plasma and urine. Findings indicate that all substances tested in hemorrhaged volunteers afforded a greater increment of circulatory volume than had been reported for unbled subjects. The results obtained with plasma, 5% oxypolygelatin solution, and saline will be compared as to their effect on plasma and blood volume. (This work was done under contract number DA-49-007-MD-107 between the University of Pennsylvania and the Department of the Army.)

Influence of heart disease on expected overshoot in blood pressure following a Valsalva maneuver. E. I. ELISBERG, E. SINGIAN AND G. MILLER (introduced by J. STAMLER). *Cardiovascular Dept., Med. Research Inst., Michael Reese Hosp., Chicago, Ill.*

An overshoot in blood pressure and a bradycardia normally occur after the straining in the Valsalva maneuver. These were found to be abolished in the presence of significant mitral stenosis, congestive heart failure, pericarditis, advanced pulmonary vascular disease, and by ganglionic blocking agents. The overshoot and bradycardia appear to be blocked by preventing the normal ingress of blood into the systemic vessels after straining. This can be produced by a significant mitral valve obstruction, a marked depression of the failing heart to raise its output, and a limitation to cardiac filling in some forms of pericardial disease. Whether a constricted pulmonary vascular bed can do this also is not settled; the lack of response in some cases may be due to a failing right ventricle. Ganglionic blocking agents prevent the pressor response (and secondarily the bradycardia) after the Valsalva maneuver by interrupting the reflex path. The blood pressure overshoot appears earlier in congenital overriding aorta since blood enters the aorta directly from the right ventricle.

Central depression by drugs which block neuromuscular transmission. C. H. ELLIS, STATA

NORTON* AND WALLER V. MORGAN.* *Wellcome Research Labs., Tuckahoe, N.Y.*

In the use of myoneural blocking agents in surgical procedures, occasional cases have been reported in which protracted respiratory arrest is encountered. Development of compounds which specifically antagonize peripheral myoneural blockage by Syncurine and *d*-tubocurarine has made it possible to demonstrate that after large doses of either of these blocking agents, respiratory arrest in cats and dogs clearly outlasts the period of neuromuscular block. In general, with *d*-tubocuraine and Syncurine, those animals which showed failure of respiration exhibited a definite lag in the return of spontaneous respiratory movements after the diaphragm was again responsive to electrical stimulation of the phrenic nerve. Diacetylcholine also showed this phenomenon in 3 of 11 instances, but in 2 cases in which respiratory arrest was complete, spontaneous respiration began simultaneously with return of the diaphragmic twitch. Action potentials recorded from the phrenic nerve in dogs were found to be lacking during the period of protracted respiratory arrest, but were shown to return with the restoration of spontaneous respiration. During the period between return of the phrenic-gastrocnemius twitch and the return of spontaneous respiration, lobeline, a specific stimulant of the respiratory center, caused immediate resumption of respiratory movements. The conclusion is made that the 3 neuromuscular blocking agents can show evidence of central depression in addition to their peripheral activities.

Effects of *d*-tubocurarine on blood flow and motility of mammalian intestine. LEONARD ELWELL* AND JOHN W. BEAN. *Dept. of Physiology, Univ. of Michigan, Ann Arbor.*

An isolated segment (about 12 inches below the pyloris) of dog intestine set up in a constant temperature both maintained at 38°C. was perfused under constant pressure with heparinized dog blood previously equilibrated with a mixture of 3% CO, 30% O_2 in N_2. Inflow and outflow of blood were recorded continuously by thermopile and drop methods, intestinal motility by tambours connected to each end of the segment. Intralumenary pressure was usually about 3 cm. of water. *d*-Tubocurarine previously warmed to bath temperatures was rapidly administrated by needle through the wall of the inflow connection as were also the saline controls. Doses of from about 1–4 units regularly resulted in an increased blood flow of from slight to well-pronounced magnitude, and markedly depressed the amplitude of the rhythmic contractions and tonus of the gut. These changes were occasionally preceded by an initial sharp but transient increase in tonus and amplitude of contraction during which time blood flow was temporarily reduced, frequently to complete stoppage, presumably due to vascular constriction by the increased intestinal tonus. Because of the great influence of intestinal motility and tonus on intestinal blood flow, the experimental results do not as yet conclusively demonstrate to what extent the more prolonged volume flow effects are due to the action of *d*-tubocurarine on the vessels themselves or on the intestinal musculature. The results indicate that the augmentative influence of *d*-tubocurarine on blood flow in the intestine is much less pronounced than it is on the flow striated muscle (*Am. J. Physiol.* 164: 734, 1951).

An apparent instability of conditioned and unconditioned hind leg flexion in certain conditioning situations. J. D. EMERSON (introduced by JOHN S. GRAY). *Dept. of Physiology, Northwestern Univ. Med. School, Chicago, Ill.*

Hind leg flexion to electrical stimulation of the foot was conditioned to auditory stimuli in cats, with the presentation of 100–200 conditioning trials per day. Response magnitude was measured electromyographically from skin electrodes above biceps femoris. In most of the cats the conditioned response was eliminated in the same stimulus situation in which it had been established. The unconditioned response was also reduced in magnitude in most and disappeared entirely in a few of the cats. The results are compared with those obtained when the unconditioned stimulus was given alone in the cat, with those obtained in similar conditioning situations in the dog and with those obtained in the cat in shorter conditioning periods.

Evaluation of factors in determination of cardiac output by dye dilution methods. J. ENGLEHARDT, A. E. LEWIS, R. D. GOODMAN AND E. A. SHUCK (introduced by V. E. HALL). *Pathology Sec., Atomic Energy Project, School of Medicine, Univ. of California at Los Angeles.*

Studies on rabbits by single injection and constant infusion methods reveal that: *1*) acceptable plateau during constant infusion of T-1824 can generally be obtained if artefacts introduced by measuring techniques are eliminated and effects of axial streaming minimized; *2*) the first portion of the time-concentration curve during a constant infusion demonstrates conclusively that the blood volume of the heart as well as the lung has a significant effect on the results obtained and is therefore measurable. By direct measurement of dye and extractable hematin from excised hearts and lungs it can be shown that: *1*) the loss of dye to tissues is negligible, and *2*) there is enough agreement between the direct and indirect measurements of heart and lung blood volumes to establish the validity of the indirect methods. Methods, derivations, and data will be presented. (Based on work performed under Contract No. AT-04-1-

GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.)

Effect of x-irradiation on incorporation of inorganic P^{32} into phospholipids by rat liver slices. CECIL ENTENMAN AND ERNEST O. WEINMAN (introduced by LESLIE L. BENNETT). *Biochemistry Branch, Biological and Med. Sciences Div., U. S. Naval Radiological Defense Lab., San Francisco, Calif.*

Liver slices of irradiated and non-irradiated rats were incubated in bicarbonate-Ringer's solution and the incorporation of inorganic P^{32} into phospholipids was measured. In all cases investigated, i.e. 14 and 24 hours after 2500 *r* x-irradiation and 24 hours after 1000 *r* x-irradiation, liver slices of x-ray exposed animals incorporated more P^{32} into their phospholipids than did slices of control animals. The specific activities of such phospholipid-P were also higher in the irradiated groups. Pre-radiation treatment with cysteine did not exert a significant effect on such P^{32} incorporation into phospholipids or on the specific activities of phospholipid-P when liver slices were incubated 24 hours after 1000 *r* x-irradiation. Fourteen and 24 hours after exposure, the livers of the irradiated rats used in the above investigations were heavier than the livers of their controls. The *liver weight*/body weight ratios were also greater in the irradiated rats. Such differences could be observed regardless of whether results were expressed on a liver wet weight or liver dry weight basis. Fourteen hours after 2500 *r* x-irradiation, rat livers were analyzed for their dry weights, total lipids, phospholipids and non-phospholipid fats. The percentage concentrations of these substances were the same in exposed and non-exposed animals. Since livers of irradiated rats were, however, heavier than those of controls the total amounts of these substances found in the livers were correspondingly greater in the case of the irradiated rats. But volatile materials and lipids accounted for less than 80% of the total difference in liver weight, indicating that the amounts of other constituents must also have been greater in the irradiated livers.

Hydrolysis of diphosphopyridine nucleotide by snake venom. DEAN P. EPPERSON (introduced by E. ALBERT ZELLER). *Mayo Foundation, Graduate School, Univ. of Minnesota, Rochester.*

The rapid splitting of DPN by an enzyme present in the venom of most species of venomous snakes has been studied by using the liberation of CO_2 from a bicarbonate buffer to follow the course of the reaction (CHAIN, E.; *Biochem. J.* 33: 407, 1939; ZELLER, E. A.; *Helvet. chim. acta.* 32: 2512, 1949; 33: 821, 1950). In a zinc-free medium less than 1 mole of inorganic phosphate is released per mole of DPN and no adenylic acid is produced; this suggests that DPN hydrolysis involves the dephosphorylation of the adenine moiety. In a medium containing zinc ion in 4 millimolar concentration, practically no inorganic phosphate is produced, although there is no decrease in the final yield of CO_2; neither ammonia nor pyrophosphate is produced and there is a high yield of adenylic acid. These observations suggest that the initial breakdown occurs at the pyrophosphate bond. Other considerations seem to exclude alternative sites of hydrolysis: the pyridine-ribose bond is undisturbed and the different rates of phosphate liberation by venom in the presence of various bivalent ions correspond to the behavior of venom 5-nucleotidase in similar media. The initial splitting of reduced DPN is somewhat more rapid, and the final phosphate yield greater, suggesting partial dephosphorylation of dihydronicotinamide mononucleotide. The strong inhibition of DPN pyrophosphatase by antivenom sera will be discussed.

Recording seizure occurrence in experimental epilepsy. CARL F. ESSIG AND GEORGE L. BARNARD (introduced by WADE H. MARSHALL). *Natl. Insts. of Health, Natl. Inst. of Mental Health, U. S. Public Health Service, Bethesda, Md.*

Chronic recurrent epileptic seizures occur following the application of alumina cream to the cerebral cortex of Rhesus monkeys as described by Kopeloff, Barrera and Kopeloff. A method has been devised to record the onset, frequency and temporal incidence of the convulsions. An activity cage with sheet metal walls and a floor supported from below by compression springs was constructed. The monkey's movements resulted in vertical undulations of the floor which were transmitted via an attached lever and connecting wire to an ink writing pen. The pen working against spring tension wrote on EEG paper moving 2.4 cm/min. which permitted continuous recording. Preliminary observations were made on 2 monkeys prepared with alumina cream 6 months previously. During the postoperative period these animals had never been seen to convulse spontaneously or in response to administered stress. It is indicated from the activity records obtained that both animals are subject to spontaneous nocturnal convulsions. One monkey was observed 18 days during which 3 nocturnal seizures occurred. There were no diurnal convulsions during this period. The other monkey had a single nocturnal attack during 6 days of observation. In both animals the ictal episodes appeared to begin during a period of complete inactivity. However, complete immobility of the cage floor prior to a seizure does not necessarily indicate that the animal was asleep when the attack began.

Relation of dietary protein to reproduction in the rat. HERBERT M. EVANS AND MARJORIE M. NELSON.* *Inst. of Exper. Biology, Univ. of California, Berkeley.*

Normal females were placed the day of breeding on purified diets supplemented with all known vitamins and varying in protein level from 0–20%. As the protein level was decreased from 20–15% and to 10%, few adverse effects on reproduction were observed. The 5% protein level resulted in a high incidence of stillborn young together with marked decreases in birth weight of young and maternal weight gain during gestation. On a protein-free diet marked reproductive upsets occurred, namely, early resorptions or even failure of implantation. Fetal death occurred on or before the 10th day of gestation in the majority of rats. Pair-fed controls demonstrated that food restriction, although marked, (55% of *ad lib.* intake) played little or no role in these reproductive upsets. Institution of the same protein-free diet after implantation had occurred (7 days after breeding) resulted in normal implantation and 90% litters, thus indicating a critical need of protein for implantation and the early stages of fetal development.

Unsaturated fatty acids in rabbit bone marrow. JOHN D. EVANS (introduced by ESTHER M. GREISHEIMER). *Dept. of Physiology, Temple Univ. School of Medicine, Philadelphia, Pa.*

If acetate buffer at pH 5.0 and at a final concentration of 0.3 M is added to normal rabbit bone marrow *in vitro*, there is a large uptake of oxygen. This effect follows a lag of variable duration. In marrow obtained from rabbits in poor nutritive condition the oxygen consumption may be absent. Analysis suggests that unsaturated fatty acids are responsible for the oxygen uptake. When weighed amounts of linoleic acid are suspended in water with powdered hemoglobin at pH 5.0, there results an oxygen consumption which when plotted against time yields a characteristic though empirical curve. The constancy of this curve is such as to permit a semi-quantitative estimation of the linoleic acid in an uncomplicated physical system. Linolenic and arachidonic acids give essentially similar curves. It is hoped that with this method it will be possible eventually to determine more exactly the amounts of unsaturated fatty acids in rabbit marrow and thereby indicate some role for these substances in marrow metabolism.

Water diuresis in adrenalectomized rats treated with cortisone acetate and nor-epinephrine. W. J. EVERSOLE AND F. A. GIERE.* *Dept. of Biology, Univ. of New Mexico, Albuquerque.*

Previous studies have shown that while adrenal cortical hormones will stimulate the elimination of administered water in the adrenalectomized animal they will not restore the diuresis to that characteristic of the intact animal. Since nor-epinephrine and cortisone markedly affect salt and water metabolism it was considered worthwhile to study the effects of these hormones, singly and in combination, on water diuresis in adrenalectomized rats. Sprague-Dawley females were adrenalectomized and given daily doses of 0.2 mg. DCA, and 0.2 mg. cortisone acetate and 6 μg. of nor-epinephrine. At 1 and 5 days after adrenalectomy the rats were subjected to a 2-dose water tolerance test. *Group I* served as adrenalectomized controls, receiving no hormone other than the maintenance dose. Two hours prior to the test *Group II* was given 1 mg. of cortisone. *Group III* received 10 μg. nor-epinephrine just prior to each water dose. *Group IV* was given a combination of cortisone and nor-epinephrine. *Group V* consisted of sham-operated controls and received no hormone. The percentage water excreted at 3 hours for the various groups on days *1* and *5* respectively was as follows: adrenalectomized controls, 31 and 5; adrenalectomized + cortisone, 17 and 5; adrenalectomized + nor-epinephrine, 45 and 22; adrenalectomized + cortisone + nor-epinephrine, 44 and 31; sham operated, 71 and 77%. The results indicate, under the conditions of the experiment, that nor-epinephrine stimulates water diuresis in the adrenalectomized rat whereas cortisone does not. Neither of the hormones, given singly or in combinations, fully corrected the deficiencies of the adrenalectomized rat in the elimination of the administered water.

Synthesis of lipids from radioactive acetate by surviving adipose tissue slices. D. D. FELLER. *Dept. of Physiology, Tufts College Med. School, Boston, Mass.*

Inguinal adipose tissue from male Swiss mice fed an adequate diet *ad libitum* was found suitable for *in vitro* studies provided the tissue was chilled before being sliced. The slices were incubated in flasks designed for CO_2 collection in the presence of 1,2-C^{14}-sodium acetate prepared biosynthetically by the method of Barker and Kamen (*Proc. Nat. Acad. Sc.* 31: 219, 1945). Saponifiable lipids and nonsaponifiable lipids were recovered by standard procedures and C^{14} content determined in these as well as in the CO_2 fraction. Liver slices were used as a control tissue. The results obtained indicate that surviving adipose tissue is capable of synthesizing fatty acids from acetate. On the basis of either fat-free wet weight or nitrogen content, adipose tissue is considerably more active than liver. Furthermore, addition of glucose and succinate to the medium stimulates the incorporation of acetate into fatty acids for both

tissues, but the percentage increase incorporation is greater for adipose tissue. On the other hand, adipose tissue was incapable of converting acetate into the cholesterol-containing nonsaponifiable lipid fraction. Adipose tissue oxidized acetate to CO_2 at a much slower rate than liver. It appears that the fatty acids present in depot fat are derived, in part, from the carbon atoms of this 2-carbon compound by active synthetic processes occurring in the adipose cell.

Blood-clotting studies following internal radiation. JOHN H. FERGUSON, G. A. ANDREWS* AND MARSHALL BRUCER.* *Physiology Dept., Univ. of North Carolina, Chapel Hill, and the Med. Div., Oak Ridge Inst. of Nuclear Studies, Oak Ridge, Tenn.*

'Prothrombin consumption tests' by both the 2-stage and 1-stage methods were run on dogs receiving intravenous radio-gold (Au^{198}). Whole blood clotting-times, tests for circulating anticoagulant, etc. were also performed, together with routine hematological observations (including platelet counts) and general observation of the animals. It was found that the prothrombin consumption tests (especially the 2-stage) gave early and progressive evidence of radiation injury. The data will be discussed from the viewpoints of *a*) correlation with thrombocytopenia and *b*) possible nature of the clotting factors involved. It may be concluded that the plasma thromboplastic mechanisms are most sensitive to irradiation effects and that the prothrombin consumption test measures, for practical use, the 'coagulation reserve' with respect to these mechanisms.

Effect of blood infusion on left ventricular pressure and stroke volume in the closed chest dog. THOMAS B. FERGUSON,* OSCAR W. SHADLE,* AND DONALD E. GREGG. *Dept. of Cardio-Respiratory Diseases, Army Medical Service Graduate School, Washington, D. C.*

Twenty-eight determinations of cardiac output were made simultaneously by the dye dilution method and the pressure pulse contour method in 9 anesthetized dogs. Dye curves were taken with a modified photoelectric dye recorder. Phasic pressure tracings were optically recorded from the carotid artery for pulse contour outputs, from the intrapleural space, and the left ventricle. The latter tracings were obtained by needle puncture of the heart at the apex, which had previously been sutured to the chest wall. The pressure corresponding to a point on the ventricular curve after atrial systole and before ventricular systole, corrected for intrapleural effect, was used as the effective end diastolic pressure. The mean effective diastolic pressure was obtained by averaging the pressures for all the beats during each dye output period. Experimental conditions were altered from control levels by infusion of warmed, matched blood at rates from 0.5 to 5 cc/kg/min. Stroke volumes calculated by each output method agreed directionally, and before infusion varied from 9.4 to 29.3 cc., during infusion from 4.6 to 25.0 cc. The ventricular diastolic pressures before infusion ranged from 2.8 to 12.1 mm. Hg, during infusion from 1.1 to 38.1 mm. Hg. During any one infusion the heart rate, stroke volume and effective end diastolic pressure, each compared to its control value, varied independently and without predictable directional change. No consistent relationship was found between the stroke volume and the end diastolic pressure in the left ventricle.

Irreversible shock and myocardial failure developing in dogs following ligation of the inferior vena cava. ANDREW O. FITZMORRIS AND WILLIAM F. FRITZ (introduced by ROGER K. MCDONALD). *Clinic of Gen. Medicine and Experimental Therapeutics, Natl. Heart Inst., Bethesda, and Cardiovascular Clinic, U.S. Public Health Service Hospital, Baltimore, Md.*

Following ligation of the supra-diaphragmatic inferior vena cava in dogs, a type of circulatory failure develops, which, to our knowledge, has not previously been noted. Following ligation of the inferior vena cava above the liver, the arterial blood pressure falls immediately to levels incompatible with life. This cannot be reversed by the immediate infusion of whole blood or saline, regardless of the amount employed; in this respect it differs from the post-hemorrhagic shock syndrome. In addition to the immediate drop in arterial blood pressure, there is progressive myocardial failure as evidenced by a steady rise in right and left auricular pressures, cardiac dilatation, and finally cardiac arrest. This sequence of events is observed whether or not fluids are infused. In various shunt experiments thus far performed, it appears that occlusion of the hepatic circulation may be the most important factor in the production of this type of shock. Studies designed to test this hypothesis are now in progress.

Body temperature a major factor in drowning survival. RUTH H. FODEN,* FRED W. STEMLER,* WM. T. ROCKHOLD* AND WM. A. HIESTAND. *Lab. of Animal Physiology, Dept. of Biological Sciences, Purdue Univ., Lafayette, Ind.*

In an investigation of the effect of altering the body temperature of mice on survival to drowning, a significant linear relationship appeared when body temperatures were plotted against survival time as measured by the final gasp. Various agents as well as mechanical means of chilling in ice baths and heating with infra-red radiation were used to alter internal temperature. Substances which markedly lowered temperature al-

ways caused an increase in survival time; those which increased temperatures or metabolic activity caused a decrease. The most pronounced increase in survival occurred with chilling, previous exposure to oxygen, injections of ethyl alcohol, India ink, and powdered charcoal; prolongation to a lesser degree was shown by ACTH, cortisone, thiouracil, and gum acacia. A decrease in survival time followed heating, exposure to cold for 48 hours, dinitrophenol, and, to a lesser degree, thyroxin. Other substances tested,such as cocaine and histamine, showed no appreciable effect. Exercise (swimming in water for periods of 15 minutes to one hour) increased survival time by over 20%. Starvation for 48 hours reduced survival time only slightly. All animals were males obtained from Carworth Farms. (Aided by a grant from the Office of Naval Research.)

Effects of epinephrine and nor-epinephrine on coronary blood-O_2 exchange. E. L. FOLTZ, J. W. WEST, H. E. LESSNER, P. H. BULLE AND M. E. HESS (introduced by D. M. AVIADO, JR.). *Lab. of Pharmacology and Dept. of Medicine, Univ. of Pennsylvania, Philadelphia.*

In intact dogs under morphine and a combination barbiturate anesthesia (Dial, urethane, pentobarbital), catheterization of the coronary sinus was accomplished under fluoroscopic guidance for sampling coronary venous blood. Following the injection of epinephrine and nor-epinephrine in doses of 1–100 gamma/kg. approximately similar rises in coronary venous oxygen saturation were noted. The observed change was of short duration and occurred with the maximal rise in blood pressure and a reflex slowing of the heart. A dose of 5 gamma/kg. of either drug produced a mean rise in the coronary venous O_2 saturation of approximately 35% which was highly significant in a small series of experiments. The effect was partly blocked by atropine administered shortly before the control observations; although atropine prevented slowing of the heart rate at the time of the maximal increase in pressure, the cardiac rates before the injection of these drugs was much higher than the 'basal' levels provided by our anesthesia. In conclusion, under the conditions of these studies, there appears to be no essential difference between the actions of epinephrine and nor-epinephrine on coronary blood-O_2 exchange, arterial blood pressure and cardiac rate. It would appear that an augmented coronary flow initially delivered a greater O_2 supply than was utilized by the myocardium after the stimulation by these sympathomimetic drugs.

Retinal response to color shift. A. FORBES AND S. BURLEIGH.* *Biological Labs., Harvard Univ., Cambridge, Mass.*

Onset and cessation of illumination both evoke electric responses in vertebrate retinas. If with no change in effective intensity, shift from one color to another evokes electric response, it relates the source of potential to color discrimination. A beam-splitter brought 2 light beams to coincidence on the retinas of frogs or turtles. Interference filters delivered nearly monochromatic light,—red, yellow, green or blue. A beam-shifting shutter made the transition between colors with minimum flicker. Intensities were balanced with rheostats in the lamp circuits. With all 6 combinations of these colors the shift evoked electric responses; no balancing of intensities abolished it. The form of response in long wave to short wave shift differed from that in the reverse shift. In dark-adapted frogs, when the beams were equivalent as judged by the '*b*-wave' (positive), the preceding '*a*-wave' (negative) was absent or small and brief in the short-to-long-wave shift, larger in the reverse shift. The *b*-wave showed longer duration in short-to-long-wave shift than in the reverse. Frogs have both rods and cones; the above differences might relate to differential involvement of rods and cones in responses to different colors. Yellow and blue with light-adapted frogs showed reversal of the above differences. Turtle retinas (only cones) showed shift responses with less difference between short-to-long and long-to-short shifts than in the frog. The slight differences more nearly resembled those in light-adapted than in dark-adpated frogs. Rod-cone differential may therefore explain some but no all features of the responses.

Observations on CO pulmonary diffusing capacity. R. E. FORSTER, II AND W. S. FOWLER. *Depts. of Physiology, Pharmacology and Anesthesiology, Graduate School of Medicine, Univ. of Pennsylvania, Philadelphia.*

The M. Krogh method of measuring pulmonary CO diffusing capacity (*J. Physiol.* 49: 271, 1915) makes several important assumptions. We have studied the following of these: *a*) that following inspiration of a gas containing CO, all parts of the lung have the same initial P_{ACO}; *b*) that 2 successive expired alveolar gas samples, following a single inspiration, contain the same proportional contributions from all parts of the lung; *c*) that the CO diffusing capacity/lung volume is constant throughout the lung; *d*) that the pulmonary capillary blood P_{CO} is negligible. CO concentration in expired alveolar gas was measured by means of an infra red absorption detector. Uniformity of alveolar ventilation was studied by following the He or N_2 concentration in expired gas with a rapidly recording mass spectrometer. Results were as follows. Alveolar ventilation and emptying sequence were uneven so that *assumptions a* and *b* were incorrect. The alveolar CO con-

centration did not fall exponentially with time, suggesting that the diffusing capacity/lung volume varies throughout the lung and that *assumption c* was incorrect. The apparent CO diffusing capacity at $P_{A_{O_2}} \sim 40$ mm. Hg was higher than at $P_{A_{O_2}} \sim 100$ mm. Hg, suggesting that *assumption d* was incorrect, and/or the true diffusing capacity was altered. A method has been developed which takes into account uneven ventilation and varying diffusion capacity/lung volume.

Effect of cortisone on tissue protein-bound iodine and iodine content of thyroid gland. WILLIAM C. FOSTER (introduced by J. F. MCCLENDON). *Dept. of Physiology, Hahnemann Med. College, Philadelphia, Pa.*

This study was made in an attempt to explain the decrease in thyroid activity as a result of adrenal insufficiency reported earlier by the author, and the decrease produced by cortisone. Since McClendon and Foster showed a relationship between blood and tissue protein-bound iodine a similar study on cortisone was thought useful. Rats approximately 90 days old received single massive injections of cortisone acetate (150 mg/kg.). After 24 hours blood was withdrawn from the heart; the animal was sacrificed and the thyroid gland, liver, kidneys, leg muscles and heart were excised. Tissue and blood protein-bound iodine were determined by a modification of McClendon and Foster's method. The results of this study are consistent with previous findings that blood protein-bound iodine is reduced by the administration of cortisone. The tissue protein-bound iodine parallels the blood protein-bound iodine in muscle. Liver protein-bound iodine in the cortisone-treated animal was higher. This elevation may be due to the formation of an iodine-cortisone complex. The iodine content of the thyroid gland was only slightly reduced by single massive dosages of cortisone.

A method for producing uniform distribution of inspired gas. W. S. FOWLER AND R. E. FORSTER, II. *Depts. of Physiology, Pharmacology and Anesthesiology, Graduate School of Medicine, Univ. of Pennsylvania, Philadelphia.*

Certain methods for measuring pulmonary diffusing capacity and blood flow assume or require uniform distribution of an inspired foreign gas to all alveolar spaces. Gas distribution was studied in normal subjects by continuous analysis of He (rapid analysis mass spectrometer). Results follow: *1*) In a maximal expiration following a single maximal inspiration of 80% He—20% O_2, alveolar gas expired early contained a greater He concentration than alveolar gas expired late. *2*) If a small volume (several hundred ml.) of He—O_2 was inspired and followed without inspiratory interruption by air (remainder of vital capacity), alveolar gas expired early contained a lower He concentration than that expired late. *3*) By varying the proportions of inspired He—O_2 and air, practically uniform expired He concentrations can be obtained. *Observation 1* indicates that distribution was uneven and that the proportion delivered from differently ventilated regions changes during expiration, i.e. sequential emptying. Since the emptying sequence was similar in *1*, *2* and *3*, it appears that inspiratory distribution was affected in *2* and *3*, and was practically uniform in *3*. This conclusion is supported by finding practically uniform expired concentrations after He—air inspirations as in *3*, despite changing the emptying sequence by *a*) expiring in one lateral recumbent posture after inspiring in the other (DuBois), and *b*) expiring rapidly following a normal partial expiration.

Electrolyte and water shifts in human muscle during in vitro edema. CHARLES L. FOX, JR., AND SIGMUND LASKER.* *New York Med. College and Flower-Fifth Avenue Hosp., New York City.*

Edema is usually regarded as the accumulation of extracellular fluids in tissue spaces. Our investigations (*Am. J. Med. Sc.* 216: 1, 1948; *Pediatrics* 7: 186, 1951; *Ann. Surg.* 114: 626, 1951) suggested that the tissue cells may also be involved. *In vitro* experiments were performed in which human rectus muscle excised at operation was promptly immersed in a synthetic extracellular fluid (200 mg. tissue in 1.0 ml. fluid) at 22° or 37° C. and aerated with 95% O_2 + 5% CO_2. After immersion from 2–60 minutes, muscle and fluid were analyzed separately. The greatest change occurred within 2–10 minutes when concentrations of Na and CO_2 in the fluid had decreased by 10–15 mEq/l. and K had increased from 5–16 mEq/l. The Cl concentration remained unchanged after 60 minutes at which time slightly greater changes in the other ions had occurred. The muscle gained water (plus 6%), Na and Cl (which doubled) and lost up to one-half its K. The muscle electrolyte changes continued in these directions beyond the great initial change at 2–10 minutes. In these experiments the muscle acquired from the extracellular phase more Na than water but as much Cl as water. This is not in accord with a movement of extracellular fluid as a unit into the tissue spaces. Although doubts exist as to the viability of such excised tissues the similarity of these tissue changes to those found after tourniquet trauma *in vivo* and after death during the nephrotic syndrome suggest that these *in vitro* changes may not be unrelated to formation of edema *in vivo*. The Na, Cl, CO_2 pattern of the extracellular fluid after immersion resembles that seen in the plasma of many patients with cardiac or nephrotic edema; this may be significant.

An automatic reaction time and performance indicator. WILLEM S. FREDERIK (introduced by BENJAMIN G. FERRIS, JR.). *Dept. of Physiology, Harvard School of Public Health, Boston, Mass.*

A performance indicator has been developed to facilitate the analysis of reaction time measurements used in the quantitation of fatigue. The instrument is basically an automatic time measuring device. The subject being tested receives signals consisting of light flashes at automatically controlled irregular time intervals; the response consists of button pushing or other selected motor activity. Into a series of vertical plastic tubes, steel balls are dropped at the moment of the reaction to the stimulus. Every tube represents a certain reaction time; the time difference between two adjacent tubes is 0.02 seconds. The total number of balls in each tube after the test is finished gives the frequency distribution of reaction times. This frequency distribution as a rule is not a normal one. The device, used as a performance indicator, is designed to be used while the man is performing his regular activities. A specific change in the pattern formed by the balls in the plastic tubes indicates a deterioration in the performance of the work. Errors are also registered automatically. The instrument has a definite application in the field of applied physiology as an objective indicator of fatigue.

Distribution of administered Rb^{86} carbonate in mouse, guinea pig, dog and man. A. STONE FREEDBERG, H. BENAIM PINTO AND ALBERT ZIPSER (introduced by HERRMAN BLUMGART). *Yamins Research Lab., Beth Israel Hospital, and Dept. of Medicine, Harvard Med. School, Boston, Mass.*

Considerable interest has recently been evinced in trace elements found in animal and man. The present report describes the clearance from blood, the urinary and fecal excretion and the ditsribution and turnover of administered Rb^{86} carbonate (obtained from Oak Ridge) studied in the guinea pig, mouse, dog and man. Clearance from the blood, studied in man and dog, showed less than 5% remaining in the total circulating blood 30 minutes after intravenous administration. In man the red cells show a maximal uptake within 30 minutes and maintain this concentration despite a rapidly diminishing plasma level. Blood uptake at 24 hours was lower than that of any tissue examined except for brain; the plasma concentration represented only a small fraction of blood content. In each species examined, uptake at 24 hrs/gm. of tissue, expressed in percentage of dose administered, was highest in the pancreas, with decreasing concentrations in the spleen, liver, lymph node, testis, duodenum, small bowel, striated muscle, heart, adrenal, kidney, lung and ovary. The renal excretion rate (Keating, Berkson) is significantly greater in the guinea pig than in the dog and man. Fecal excretion is small in all species. In each species the brain Rb^{86} uptake was smaller than any other tissue examined and was below blood concentration; in the dog the uptake by brain cortex was lower than that of underlying white matter. In mice with implanted Sarcoma 37, astrocytoma and neuroblastoma, the uptake by the tumour was respectively 1.5, 4 and 10 times that of normal brain. In one patient with a brain metastasis from a carcinoma of the esophagus, the uptake in the metastasis was 17 times that of normal brain. The ratio of tumour uptake to that of normal brain was highest 2–4 hours after administration. The turnover in and radiation delivered to the pancreas, liver, spleen and muscle will be described.

Effect of carbonic anhydrase inhibitor 6063 (2-acetylamino - 1,3,4 - thiadiazole - 5 - sulfonamide) on renal excretion of fluid and electrolytes in patients with congestive heart failure. CHARLES K. FRIEDBERG AND MARK HALPERN (introduced by MILTON MENDLOWITZ). *Mount Sinai Hosp., New York City.*

Since abnormal renal retention of sodium is a cardinal feature of congestive heart failure, patients with this syndrome were given carbonic anhydrase inhibitor 6063 in order to inhibit the renal mechanism for acidification of the urine and thus reduce the related tubular reabsorption of sodium. Six patients in various degrees of heart failure were studied. The sodium intake was between 100 and 200 mg. daily in 4 of the patients and between 1 and 2 gm. in the other 2. The fluid intake was maintained at a constant rate of 250 cc/hour. Following several control periods, 6063 was infused intravenously in doses of 5–10 mg/kg. in a period of 30 min. After the infusion of 6063, the plasma CO_2 combining power fell slightly. The urine became alkaline by the time the infusion was completed and the pH of the urine remained between 7.5 and 7.9 for 4–8 hours. The urinary excretion of titratable acidity and ammonia was abolished. The urine flow increased from a mean of 1.1 cc/min. to a mean of 4.9 cc/min. The sodium excretion increased from a mean of 11 μEq/min. to a peak of 160 μEq/min. in the 4 cases with very restricted sodium intake, and from a mean of 47.8 μEq/min. to 217 μEq/min. in the 2 cases with less stringent sodium restriction. Potassium excretion increased from a mean of 43.1 μEq/min. to 206 μEq/min., phosphate[s] from 207 μEq.–461 μEq. Chloride excretion increased relatively much less than that of other electrolytes, from a mean of 33 to a mean of 66 μEq/min. The electrolyte and fluid diuresis usually reached a peak within 90 minutes after beginning the infusion of 6063 and persisted for at least 5

hours. There was a mean weight loss of 3 pounds (range 1.5–6.0 pounds) within 24 hours.

Thyroxine-like compounds in iodinated protein and thyroid gland. W. FRIEDBERG* AND E. P. REINEKE. *Dept. of Physiology and Pharmacology, Michigan State College, East Lansing.*

The component iodinated compounds of thyroidally active iodinated casein and thyroid gland have been studied. Iodine-131 was used in the iodination of the casein and also administered to rats by subcutaneous injection. The iodinated casein was hydrolyzed with 40% barium hydroxide and the thyroid glands with 2 N sodium hydroxide. Both hydrolysates were acidified and extracted with normal butanol. Further separation was accomplished by treating the alcoholic extracts with an alkaline wash solution. Paper partition chromatography and radioautograms of the unwashed iodinated casein extract revealed the presence of at least 10 iodinated compounds, including iodide, monoiodotyrosine, diiodotyrosine, diiodothyronine, thyroxine and possibly triiodothyronine. Six of the 10, including the first 3 mentioned above, were markedly reduced in concentration by the washing procedure. The concentrations of thyroxine, diiodothyronine and possibly triiodothyronine were not noticeably affected by the alkali wash. In alkali-washed extracts of rat thyroids, non-thyroxine iodinated compounds were found in relatively smaller amounts than in similarly treated iodinated casein. Radioactivity measurements of iodinated casein extracts revealed that the iodine content was reduced 52% and 8% respectively by the first and second washings. Direct radioactivity determinations of the compounds separated on paper chromatograms showed that iodinated compounds other than thyroxine contributed significantly to the iodine content of the alkali-washed extract of iodinated casein. In the case of thyroid gland this source of error in thyroxine determinations by the butanol extraction technique is relatively less.

Effects of relaxin upon decidual reactions in the rat. EDWARD H. FRIEDEN AND JOSEPH T. VELARDO (introduced by F. L. HISAW). *The Biological Labs., Harvard Univ., Cambridge, Mass.*

The injection of 60-150 GPU/da of relaxin has been found to inhibit the decidual reaction which normally follows traumatization of the uterus of the castrate, pseudopregnant rat given progesterone. Smaller amounts of some relaxin preparations enhance, rather than inhibit, the action of progesterone. The relative inhibitory effects of various relaxin preparations from the ovaries of pregnant sows and from pregnant rabbit serum are proportional to their relaxin content over a 100-fold range of specific activity (4-450 GPU/mg.), while extracts of ovaries of non-pregnant sows are without effect. Upon heating, the potentiating effect disappears, and the inhibitory activity of preparations assaying 100 GPU/mg. or less is increased several fold. More active preparations (300 GPU/mg.) do not enhance the action of progesterone, and the effects of heating are much less marked. Like relaxin, the inhibitor is inactivated by reducing agents and acid methanol. The effects of nitrous acid and acetic anhydride are qualitatively similar to those of heating. Inasmuch as relaxin is stable to heat, and is not inactivated by deamination or acetylation, it is concluded that the inhibition is due to relaxin and not to an impurity. Application of the decidual reaction as a test response for the quantitative assay of relaxin has been found to be a useful supplement to the guinea pig assay. (Supported by a grant from the National Institutes of Health, U. S. Public Health Service, to F. L. Hisaw.)

Assay of gastric secretory depressants in the unanesthetized cat. M. H. F. FRIEDMAN, H. HOCHSCHILD* AND J. B. CARPENTER.* *Jefferson Med. College, Philadelphia, Pa.*

A two-dose histamine test on the unanesthetized cat has been developed for determination of the comparative potencies of gastric secretory depressants. The animal is provided with a gastric cannula to which is attached a non-spilling collecting flask. When fasted 16 hours the gastric mucosa usually shows an alkaline reaction and there is a low-rate basal secretion of alkaline fluid. Infrequent occurrence in the cat of regurgitation of intestinal content obviates the need for a gastric pouch. Contamination of gastric content by small amounts of saliva presents no problem except when the test drug excites excessive salivation. The trained animal sits quietly without restraint. Following a control period, histamine is administered in a dose previously determined to give approximately 50% maximum response. This is repeated 2 hours later. Test substances are given from 30 to 60 minutes before the second histamine injection, depending on their nature. The inhibitory potency of a drug is calculated in the usual manner by comparing in test and control experiments the total output of acid for the hour following the second histamine with that following the first. Frequent standardization of the histamine response and intervals of at least 6 days between consecutive tests on each cat are necessary. Seventeen cats have been used by us, some animals for as long as 2 years, with satisfactory results. Data on the influence of potency of various drugs to depress gastric secretion will be presented.

Mechanism underlying hypercholesteremia after injection of Triton. MEYER FRIEDMAN AND SAN-

FORD O. BYERS. *Harold Brunn Inst., Mount Zion Hospital, San Francisco, Calif.*

Injection of Triton A-20 induces hypercholesteremia in the rat by causing an elevation of cholate in plasma. The intravenous injection of 0.6–1.0 cc. of Triton (10% solution) into 8 rats was followed within 1 hour by a rise of plasma cholate to an average level of 18.8 mg/100 cc. Following this rise, plasma cholesterol was observed to increase so that at the end of 4 hours it had risen from 58–106 mg/100 cc. In subsequent experiments, it was found that Triton causes elevation of plasma cholate in part at least by diminishing its hepatic excretion. The degree of 'hypercholatemia' produced in Triton-injected rats could be increased or decreased respectively by feeding of cholate or by removal of cholate by cannulation of the bile duct of the injected rats. Such changes in plasma cholate were followed by changes of plasma cholesterol in a similar direction. In view of the fact that *1*) hypercholesteremia in Triton-injected rats followed and varied in its degree directly as the cholate rise in plasma, and *2*) that the same degree of hypercholesteremia can be duplicated in normal rats by elevation of plasma cholate to a similar level by simple injection of cholate, it appears that hypercholesteremia observed after Triton injection stems from the hypercholatemia initially produced. Hypercholesteremia then is a secondary not a primary phenomenon after injection of Triton.

Effect of Dextran on renal function in dogs subjected to hemorrhagic hypotension. HERMAN F. FROEB,* RUBIN J. JUSTER,* CONRAD L. PIRANI,* C. F. CONSOLAZIO* AND RAYMOND C. INGRAHAM. *Army Med. Nutrition Lab. and Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.*

The following experiments were conducted to investigate any possible deleterious effects of the plasma substitute, Dextran, on the renal function of trained, unanesthetized dogs after a period of hemorrhagic hypotension (40 mm Hg for 1 hour). The blood removed was replaced in 7 dogs with Dextran and in 5 with plasma (controls). Observations of C_{Cr}, C_{Ur}, C_{PAH}, and Tm_{PAH} were established prior to the bleeding procedure and were repeated on several occasions up to the fourteenth day thereafter. The substances used for clearance studies were administered subcutaneously following hydration. The GFR (C_{Cr}), C_{Ur}, and ERPF (C_{PAH}) increased following hypotension in both groups with a decrease in the filtration fraction. The data on the transfer maximum of PAH, which was performed in 2 dogs treated with plasma and in 2 treated with Dextran, revealed an increase in the Tm_{PAH} following hypotension in the plasma group, but a decrease in the Dextran group. The decrease in the Dextran group persisted only to the fifth or ninth day post hypotension. During this time interval there was a concomitant depression in the serum proteins which was present only in the Dextran group. The limited number of dogs studied thus far as well as difficulties encountered in regulating the load/Tm_{PAH} ratios following hypotension, prevents formulation of any definite conclusions at this time.

Endocrine influences upon cytology of rat spleen. GEORGE J. FRUHMAN* AND ALBERT S. GORDON. *Dept. of Biology, Washington Square College, New York Univ., New York City.*

Giemsa-stained slides of spleen cell suspensions were counted differentially and the following values (means ± standard errors) obtained for 9 normal, adult male rats of the Sprague-Dawley strain: lymphocytes (88.7% ± 1.2); plasma cells (0.72% ± 0.2); monocytes and macrophages (3.67% ± 0.6); neutrophilic granulocytes (1.70% ± 0.5); eosinophils (0.28% ± 0.1); nucleated erythrocytes (2.76% ± 0.5); stem cells or hemocytoblasts (1.53% ± 0.5); mitoses (0.43% ± 0.1). Following hypophysectomy, the only significant alteration detected was a decrease in the percentage of nucleated erythrocytes (1.0% ± 0.3). Eosinophils were also estimated in spleen cell suspensions using the Randolph stain and hemacytometer method. These studies were supplemented with observations of Giemsa-stained spleen prints and spleen sections stained with chromotrope 2*R*. Cortisone-treated animals (1–3 mg. subcutaneously) showed significant decreases in the numbers of splenic eosinophils after 6 hours. There was a high correlation (+0.82) between the numbers of eosinophils in the spleen and those in the peripheral blood of both control and cortisone-treated rats. The eosinophils were found largely in the red pulp and were often seen in clumps of up to 15 cells in both control and experimental animals. These findings contribute to the evidence that the peripheral eosinopenia induced by cortisone in the rat is not due to sequestration of eosinophils in the spleen. (Supported by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.)

Mechanism of vasodilatation in the hind limb of dogs. M. J. FRUMIN, S. H. NGAI AND S. C. WANG (introduced by WILLIAM L. NASTUK). *Depts. of Anesthesiology and Physiology, Columbia Univ., College of Physicians and Surgeons, New York City.*

Dogs were anesthetized with a pentobarbital-Dial-urethane mixture and the vagi were divided bilaterally. A heparinized donor dog was used to cross-circulate the recipient animal via the femoral arteries and veins in order to maintain a stable perfusing pressure to the hind limb. The collateral

arterial supply to the limb was ligated. The rate of femoral arterial blood flow was recorded with a calibrated recording rotameter. The systemic arterial pressure in both animals was recorded from the brachial arteries using mercury manometers. Marked hypotension was produced in the recipient by electrical stimulation of the carotid sinus nerve, the central end of the divided vagus nerve or the depressor area of the medulla oblongata. Concomitant with this hypotension, the rate of femoral arterial blood flow invariably rose sharply, sometimes as much as 100%. This rise in flow rate was abolished entirely by excision of the ipsilateral lumbar sympathetic ganglia. The vasodilatation noted in the limb in these experiments must therefore be interpreted as the result of changed sympathetic outflow. The dorsal roots apparently do not transmit impulses centrifugally under these conditions even though direct electrical stimulation of the peripheral end of the sectioned lumbar dorsal roots does yield dilatation. Atropine injected into the femoral artery prevents the dilatation elicited by intra-arterial administration of acetylcholine but not that produced reflexly when the sympathetic nerves are intact.

Effects of beta and x-irradiation on circulation in hamster cheek pouch (motion picture). GEORGE P. FULTON, BRENTON R. LUTZ, DAVID L. JOFTES* AND FREDERICK W. MAYNARD,* *Dept. of Biology, Boston Univ., Boston, Mass.*

The vascular effects of beta-emitting glass beads implanted in the hamster cheek pouch were cinephotomicrographed. A marked increase in vascularity developed adjacent to Sr^{90} beads, but not at the site of control nonradioactive beads. Dense capillary networks and tortuosity appeared, indicating endothelial proliferation. Within 24 hours, high activity beads (140 μc.) produced increased vascularity, platelet and leukocytic thromboembolism, petechial hemorrhages confined to postcapillary venous junctions, and edema. Capillary petechiae were not found, except at junctions with venules. The arterioles were frequently narrow and beaded in appearance. At 72 hours, necrosis and infection were evident around the bead. Asynchronous fibrillation of individual skeletal muscle fibers was recorded. By 30 days, low activity beads (1 μc.) produced increased vascularity, but severe effects were not seen even with the bead implanted for 3 months. Total body irradiation with approximately LD_{50} doses (1200 r) produced white thromboembolism and petechial hemorrhages in transillumined cheek pouches. Immobilized leukocytes paved the endothelium of numerous venules, but increased adhesiveness did not correlate with the period of reduction in blood count. Within 12 hours before death, the erythrocytes frequently circulated in loose aggregates. Motion picture sequences show aggregates resolving readily into individual cells at capillary origins, without formation of occluding thrombi, and reaggregating of erythrocytes in venules after circulation through capillaries. Portions of the cheek pouch were irradiated at the site of glass beads previously implanted for identification of the irradiated area. Vascular effects from partial cheek pouch irradiations were obtained only at high doses (25,000 r). (Supported by a contract with the U. S. Atomic Energy Commission.)

A reversible biochemical lesion in stored erythrocytes. BEVERLY WESCOTT GABRIO* AND CLEMENT A. FINCH. *Dept. of Medicine, Univ. of Washington School of Medicine, Seattle.*

Whole blood stored at 4° C. in a preservative of acid-citrate-glucose has been observed to undergo changes in carbohydrate metabolism. The initiating factor is unknown, but such phosphorylated compounds as ATP have assumed an important role. During storage there is a decrease in erythrocyte ATP and other organic phosphate compounds and an increase in cellular inorganic phosphate with only a slight increase in plasma phosphate. By studying the changes in a uniformly aged population of cells *in vivo*, and by studying storage effects of tagged cells of various ages, we have concluded that the storage abnormality of the erythrocytes has no relation to cell senescence *in vivo*. Further studies were undertaken to determine the reversibility of the storage lesion. In a typical experiment, a recipient rabbit, whose erythrocyte population was labeled with Fe^{59}, received 16-day-stored rabbit erythrocytes by exchange transfusion so that 67% of the mixed population were the stored cells. It was found that the erythrocyte was able to rapidly regenerate ATP so that within 30 minutes after the exchange transfusion the ATP-P had increased from 7.1 mg/100 ml. erythrocytes to 14.6 mg. In addition, other organic phosphate compounds increased similarly, the cellular inorganic phosphate decreased markedly to the normal level, and the osmotic fragility of the erythrocytes returned to normal. A similar reversibility of this storage lesion could be shown in a human subject. A comparison was made of the effect of fresh plasma and synthetic media with the exchange transfusion procedure. Such *in vitro* manipulations did not have any appreciable effect on reversing the biochemical lesion of storage and did not improve subsequent *in vivo* survival of the cells. However, a short term 'rejuvenation' of stored cells in the circulation of another animal appeared to mitigate storage damage and extend survival.

Effect of change of posture on resting level of lungs and expiratory reserve volume in patients with emphysema. MORTON GALDSTON AND IRIS WEISS.* *Research Service, Third (NYU) Med. Div., Goldwater Memorial Hosp., Welfare Island, N. Y.*

The influence of change of posture from supine to sitting on the resting level of the lungs (functional residual capacity) and expiratory reserve volume was studied in 6 patients with chronic pulmonary emphysema. It has been reported that in healthy adults the increase in expiratory reserve volume accounts for only about 55% of the approximately 1 liter rise in functional residual capacity on changing from supine to sitting. In these patients, in contrast, on changing from supine to sitting, functional residual capacity increased not at all or only 100–350 cc. although the predicted average increase for these patients was approximately 600 cc. Furthermore, in nearly every instance the increase in expiratory reserve volume was 100–300 cc. more than the increase in functional residual capacity on going from supine to sitting. These results suggest that some patients with chronic emphysema develop more forceful use of expiratory muscles as a partial compensation for decrease of pulmonary elasticity.

T deflection of ventricular heart muscle strips. SOLOMON GARB (introduced by MCKEEN CATTELL). *Dept. of Pharmacology, Cornell Univ. Med. College, New York City.*

It has been shown that the T deflection of the isolated cat papillary muscle is normally in the same direction as the R, and that it is depressed and inverted by injury, anoxia and rapid rates. This suggested that either the present concepts of the genesis of the T deflection are incorrect, or that some peculiarity in the papillary muscle makes it act differently from other types of myocardial strips. Therefore, a series of experiments was performed on 11 strips, cut from the cat left ventricle in the usual fashion. They were observed for many hours, while they presumably recovered from the initial cutting injury. Initially, most electrograms had inverted T deflections. However, after recovery periods of from ½–23 hours, 8 of the 11 strips developed upright T deflections which were then depressed and inverted by anoxia and rapid rates of stimulation, and which returned to their original configuration when control conditions were restored. Since the T deflection is normally upright in a simple strip which has partially recovered from injury it is unlikely that its genesis can be adequately explained by membrane repolarization alone. Since the T deflection of heart muscle and the after-potenials of nerve are similar in several respects, it is possible that the T deflection, like the nerve after-potentials, is produced by metabolic processes.

Effect of cord transection and dorsal root interruption on oscillating potentials of the cat's spinal cord. EDGAR L. GASTEIGER AND VERNON H. MARK (introduced by PAUL O. CHATFIELD). *Dept. of Neurosurgery, Massachusetts General Hosp., and the Dept. of Physiology, Harvard Med. School, Boston, Mass.*

The effect of low thoracic spinal cord transection on the oscillating potentials in the distal segment varied with changing experimental conditions. In 80% of a series of etherized cats there was a marked increase in amplitude of both high and low frequency components immediately after transection. Striking changes were not observed in series of animals mildly and profoundly narcotized with barbiturates. The addition of curare to the above agents had no observable effect. The profound depression of oscillating potentials immediately after transection, as reported in the literature, was not observed under the experimental conditions and anesthetics used. A further isolated spinal cord preparation was made by proximal (T-10) and distal (L-5) cord transection and dorsal root interruption of the isolated cord. The amplitude of the oscillating potentials of such a segment was reduced up to 80% of control values, but oscillating potentials persisted over 6 hours after isolation in the acute preparation. Gross sensory stimulation through a single remaining dorsal rootlet caused an increase in the amplitude of the oscillating potentials, which, in some cases, exceeded the control recording. After this rootlet had been severed, the most vigorous sensory stimuli in the involved dermatomes caused no change in the electrical activity of the isolated spinal cord segment. Chronic experiments, done weeks after isolation will be reported on. This preparation will have histological checks to control the effect of injury potentials and reduced blood supply.

Relative effectiveness of different esters of desoxycorticosterone. ROBERT GAUNT, JAMES H. LEATHEM, CONSTANCE HOWELL* AND NANCY ANTONCHAK.* *Research Labs., Ciba Pharmaceutical Products, Inc., Summit, N. J.*

Various actions of the compounds listed below were observed in adrenalectomized rats. The compounds were given as a single subcutaneous injection either in oil or in microcrystalline aqueous suspension (average crystal size 30–50 μ, except cortisone, 5–10 μ) at the time of adrenalectomy. For brevity only survival data are detailed on immature male adrenalectomized rats —7–16 rats/group—at 1 of the dose levels (1 mg.) tested.

COMPOUND	SURVIVAL IN DAYS Oil	Aq. Susp.
Desoxycorticosterone	8.9	12.8
DC acetate		
DC trimethylacetate[1]	9.8	>13.0
DC phenylacetate[2]	18.4	>25.0
DC-β-cyclopentylpropionate[2]	10.0	>19.3
Cortisone acetate	12.7	11.6
Compound S acetate		8.8
Controls—untreated		12.0
	7.1	6.4

Prepared by: [1]Wieland, Heen, Schmidlin and Miescher. [2]Dr. A. St. Andre.

In oil solution the duration of activity of the trimethylacetate clearly exceeded that of other compounds. In aqueous suspension, the effects particularly of the trimethylacetate and phenylacetate were greatly prolonged, permitting the development of adrenal accessories in nearly half of the cases, and preventing accurate calculation of survival data. Gross and Tschopp (in press) also observed prolonged activity of the trimethyl compound, as have Swingle *et al.*, for both the trimethyl- and phenylacetate. As expected, cortisone acetate was much the most active of the compounds in suppressing ACTH release (prevention of compensatory hypertrophy) and in maintaining water diuresis. It did not, however, reduce chloride excretion during water diuresis tests as did the other compounds.

Adrenal changes associated with a low protein diet. HERBERT GERSHBERG* AND ELAINE P. RALLI. *Dept. of Medicine, College of Medicine, New York Univ.-Bellevue Med. Center, New York City.*

It is established that a low protein-high fat diet will produce fatty infiltration of the liver. Other tissues probably also suffer from this protein deficiency, in particular the pituitary which secretes protein hormones. In rats fed such a diet (9% protein and 38% fat), the adrenals and the thymus were weighed and determinations were made of the adrenal cholesterol, liver fat and liver nitrogen after periods of 8 to 60 days on the diet. The adrenal cholesterol was increased about 2 g% after only 8 to 12 days on the diet. After 54 days, the adrenal cholesterol was further elevated and the adrenal weight had diminished. These changes are in the same direction as those observed in hypophysectomized rats, though not as pronounced. The increase in adrenal cholesterol was not associated with an increase in liver, brain or serum cholesterol which in most cases was within normal limits. Administration of ACTH (2 mg. daily) caused an increase in adrenal weight and a decrease in thymus weight, indicating that the adrenals were not refractory. Vitamin B_{12} (5 mcg. daily) had a distinct lipotropic effect on the liver within a period of 7 to 12 days, but the adrenal cholesterol was not reduced.

Potassium exchange in rat tissues. J. GINSBURG (introduced by RICHARD ASHMAN). *Dept. of Physiology, Tulane Univ., New Orleans, La., and Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.*

Intravenous K^{42} disappears rapidly from the plasma of Sprague-Dawley male rats in about 4 exponential stages. The first or vascular stage is very rapid, 95% of the injected tag disappearing in 1.5 minutes. The slower 2nd and 3rd stages ending at 10 and 45 minutes are respectively related to uptake by kidney, heart and lung and to uptake by the liver, spleen and intestine. At the beginning of the 4th stage (30–40 minutes) all visceral organs are equilibrated. This latter stage represents uptake by muscle, testes, brain, etc. Muscle K*/K equals plasma at 7 hours. Unlike the report of Walker and Wilde for the rabbit (*Am. J. Physiol.* 159: 594; 163: 759), plasma K*/K does does not rise again secondarily as if by backflow of tag from the liver. Nor does liver K*/K, after first reaching plasma K*/K, then continue to rise while plasma K*/K is yet falling. Kidney K*/K, equal to plasma at 2 minutes, continues to climb to a peak about 4 times the concurrent K*/K of plasma at 5 minutes, then declines rapidly to equilibrate with plasma at 30 minutes. All the tissues analyzed contain 81% of body potassium and at 7 hours they contain 85% of the dose. The bearing of this on the calculation of total body potassium by isotope dilution is discussed.

Potentiation of hematopoietic effect of orally administered vitamin B_{12} by glandular mucoprotein of the human stomach. GEORGE B. JERZY GLASS, LINN J. BOYD AND CHESTER S. SVIGALS (introduced by STEWART WOLF). *Dept. of Medicine, New York Med. College, Flower and Fifth Ave. Hosps., New York City.*

The hematopoietic response of patients with macrocytic anemia to small doses of vitamin B_{12} administered orally is known to be poor or inconsistent, although the same patients usually respond well to parenteral administration. It has been postulated that this discrepancy was due to the absence of Castle's intrinsic factor from the stomach of patients with pernicious anemia and the consequently deficient absorption of B_{12} from the gastrointestinal tract. In earlier studies the authors showed that one of the components of gastric dissolved mucin, glandular mucoprotein from fundal glands of human stomach closely

resembled intrinsic factor in physical and physiological properties. Moreover, this substance was found consistently absent or present only in traces in gastric juice of patients with pernicious anemia. In 7 patients with this disease oral doses of 10–30 mcg. B_{12} daily were uniformly ineffective. When 50–200 mg. of glandular mucoprotein daily was added to B_{12}, however, a full hematopoietic response occurred. Recently 2 patients with nutritional macrocytic anemia whose gastric juice contained a normal concentration of mucoprotein were studied. In these patients daily adminstration of 10–30 mcg. of B_{12} alone by mouth was followed by a prompt hematopoietic response to normal. These findings suggest the possibility that the presence of glandular mucoprotein in gastric juice may be required for small doses of orally administered vitamin B_{12} to be effective. Moreover, the quantitation of glandular mucoprotein in gastric juice may be of value in the differentiation of various types of macrocytic anemia capable of responding to B_{12}.

Chloromycetin Palmitate, a synthetic ester of Chloromycetin. A. J. GLAZKO, W. H. EDGERTON,* W. A. DILL* AND W. R. LENZ.* *Research Labs., Parke, Davis and Company and the Herman Kiefer Hosp., Detroit, Mich.*

The palmitic acid ester of Chloromycetin is a tasteless compound which has no significant antimicrobial activity, but when it is hydrolyzed enzymatically Chloromycetin is liberated. This hydrolysis occurs with duodenal secretions, with tissue homogenates, and with purified lipase and esterase preparations. Using isolated intestinal loops in the dog and rat, higher blood levels of Chloromycetin were observed when biliary and pancreatic secretions were present in the intestine. Blood levels and urinary excretion data in human subjects indicate a slight delay in absorption over Chloromycetin when the ester is given by mouth, but the blood levels remain elevated for a longer period of time. Satisfactory blood levels have been maintained in human subjects for periods of more than 4 days with no untoward reactions. Paper chromatographic studies indicate that the principal metabolic products found in the urine of human subjects after oral administration of the ester are the same as those found after administration of Chloromycetin.

Alteration of growth response of tissue cells in vitro in serum from partially hepatectomized rats. ANDRÉ D. GLINOS* AND GEORGE O. GEY. *Johns Hopkins Hosp. and Med. School, Baltimore, Md.*

Forty-four male adult rats were operated on in pairs, a partial hepatectomy being performed on one of the animals and a sham operation simulating partial hepatectomy on the other. Twenty-four hours after the operation the animals were bled. The sera obtained from the hepatectomized and the control animal of each pair were then compared with respect to their action on the growth of tissue cells *in vitro*. Primary liver explants from young rats 3 weeks of age as well as a normal fibroblastic strain were used as test tissues. Initiation of outgrowth, organization and morphology of the colonies and duration of growth period were used as criteria for the evaluation of the action of these sera on the cultures. It was found that blood serum from the hepatectomized animals was able to enhance significantly the growth of both the primary liver explants and the strain of fibroblasts. Quantitative data on the duration of the growth period at varying concentrations of sera from hepatectomized and control animals were obtained. The significance of these findings with respect to the nature of the alteration of the serum induced by partial hepatectomy and its meaning for liver regeneration will be discussed.

Vascular and respiratory factors in the circulation of cerebrospinal fluid in man. ELI S. GOLDENSOHN AND ROBERT F. GROVER (introduced by R. W. WHITEHEAD). *Dept. of Physiology and Pharmacology, Univ. of Colorado School of Medicine, Denver.*

Previous work from this laboratory on dogs showed that CSF pulsations at the cisterna magna coincident with the cardiac cycle were of considerable magnitude. This is also true in the lumbar subarachnoid fluid of man. Lumbar puncture in 23 subjects reveals such pulsations to average 18 mm. saline in amplitude (range 10–63) as recorded by strain gauge. In these same subjects, using the water manometer, these pulsations average only 1.4 mm. (range 0–2.5). The pulsations increase in amplitude as the intracranial pressure rises from cough, strain or jugular occlusion. During jugular occlusion the pulsations average 60 mm. saline (range 5–120 mm.) and closely resemble the contour of arterial pulse waves. Time relations between the respiratory cycle and its associated CSF fluctuations are not constant. With quiet breathing 12 of 19 subjects showed a rise in pressure associated with inspiration. In 12 of 16 subjects, during a short period of hyperpnea, the rising phase of the fluctuation also occurred during inspiration. Queckenstedt, coughing, and straining effects on the mean CSF pressure as recorded by the strain gauge differed considerably from the water manometer measurements as would be expected in a system requiring little fluid displacement. The data suggest that *1*) CSF pulsations associated with the vascular pulse are a significant factor in CSF circulation; *2*) the main force initiating these pulsations is

derived from the arterial pulse within the bony confines of the central nervous system; 3) in the resting subject the pulsations appear to exert a greater mechanical force/unit time than fluctuations associated with respiration.

Physiological significance of cellular radiosensitivity. ANNA GOLDFEDER. *Cancer Research Lab., Dept. of Hosps., New York City and New York Univ.*

The growth and metabolism of mammary tumors autogenous to inbred strains of mice and rats are being studied *in vivo* and *in vitro* before and after radiation. While one of the tumors, *A*, produced dense sheets of cells interwoven with fibrous connective tissue stroma and moderate numbers of mitoses *in vitro*, another tumor, *B*, under identical conditions, produced loose sheets of cells with no detectable stroma but exhibited greater mitotic activity and greater liquefaction of the culture medium. Metabolic studies *in vitro* revealed that *tumor A* had a lower O_2 uptake and lower rate of glycolysis than *tumor B*. The greater liquefaction of the culture medium of *tumor B* was attributed to the higher rate of glycolysis of this tumor. Larger doses of x-radiation were required for the destruction of *tumor A* than for *tumor B* when grown *in vitro*. However, the reverse was noted *in vivo; tumor B* required more x-ray dosage for total destruction. Mitotic indices revealed that recovery in *tumor B* occurred earlier and to a greater extent than in *tumor A* following medium doses of radiation. There was a significant difference in the appearance of the nuclei of the two tumors following exposure to the same dose of radiation. This will be illustrated. Data on the intermediary metabolism of these tumors before and after irradiation will be presented. An explanation of the difference in the radiosensitivity of these analogous tumors and the possible mechanisms involved will be offered.

Action of insulin on transfer of sugar across cell membrane; the common chemical configuration of sugars affected by the hormone. M. S. GOLDSTEIN,* LESTER HENRY,* B. HUDDLESTUN* AND R. LEVINE. *Dept. of Metabolic and Endocrine Research, Med. Research Inst., Michael Reese Hosp., Chicago, Ill.*

We have previously shown that insulin administration leads to the distribution of galactose in total body water. In the absence of insulin galactose distributes in 40–50% of body weight. This action of insulin occurs in the eviscerated-nephrectomized animal, which does not 'utilize' this sugar. On the basis of these data it was postulated that the action of insulin in facilitating sugar entry into muscle is exerted on the 'cell membrane' and not upon the intracellular systems of carbohydrate transformations. The present report deals with the specific nature of this insulin action. Using a variety of non-utilizable carbohydrates it can be shown that insulin increases the volume of distribution of hexoses and pentoses which possess the chemical configuration exhibited by glucose in its first 3 C atoms. Thus *d*(+) galactose, *d*(+) xylose and *l*(+) arabinose are affected by insulin. While the distribution of *d*(−) fructose, *d*(−) arabinose, *d*(+) mannose is not under insulin control. Neither is *d*-sorbitol as shown by Wick. The present data support further the view that insulin acts on a 'transfer' mechanism for sugars, and is a beginning step towards the elucidation of this mechanism. Data will also be presented on the similarities and differences between the effects of insulin and of muscular work on the entry of various sugars into the intracellular compartment.

Rapid cooling and rewarming of dogs by means of a pump-oxygenator. FRANK GOLLAN. *Fels Research Inst., Antioch College, Yellow Springs, Ohio.*

By catheterization of the right heart venous blood of 31 heparinized dogs was sucked into a gas dispersion oxygenator (LELAND C. CLARK, JR., FRANK GOLLAN AND VISHWA B. GUPTA. *Science* 111: 85, 1950), oxygenated and returned under pressure via the femoral arteries into the aorta. By insertion of a silvertube coil into the extracorporeal circuit the circulating blood could be cooled with ice water or warmed with water of 50° C. Depending on the flow rate it took 30–60 minutes to cool the animals to a rectal temperature of 20° C. and to rewarm them at the same rate. All animals above 29° C. survived, there was a rising mortality from 29–27° C. and from 27–19° C. all animals died. On rewarming, respiration returned; however, ventricular fibrillation was the cause of death below 27° C. rectal temperature. The observation that catheterized animals can be cooled to about 25° C. only (A. H. HEGNAUER, H. D'AMATO AND J. FLYNN. *Am. J. Physiol.* 167: 63, 1951) was confirmed. The results were not influenced by the rate of cooling and rewarming, by the intravenous administration of Ringer's solution and blood, or by calcium, epinephrine, *l*-arterenol, cedilanid C and procaine amide. Even if the entire cardiac output for the corresponding degree of hypothermia bypassed the heart by means of the pump-oxygenator, ventricular fibrillation occurred below 27° C., indicating that heart failure in hypothermia cannot be prevented by reducing the cardiac load. (This work was supported by a grant from the American Heart Association.)

Plasma concentration of ammonia-nitrogen and glutamine amide-nitrogen of the hepatectomized dog. EDWIN E. GORDON,* SMITH FREE-

MAN AND CHESTER J. FARMER.* *Dept. of Biochemistry, Northwestern Univ. Med. School, Chicago, Ill.*

The plasma ammonia- and glutamine amide-nitrogen were determined by the method of Archibald before and at intervals after complete hepatectomy on adult dogs. These dogs were maintained by the constant infusion of glucose (0.3 gm/kg/hr.). The survival time ranged from 24–32 hours. The average fasting plasma nitrogen concentration from ammonia and glutamine before hepatectomy were 0.03 mg. % and 0.98 mg. %, respectively. Blood samples drawn at 4-hour intervals during the first 12 hours following hepatectomy demonstrated a gradual but appreciable rise in their plasma concentration. The average values for the plasma preformed ammonia- and glutamine-nitrogen for 7 dogs 12 hours after hepatectomy were 0.46 (range 0.15–1.48) and 1.60 mg. % (range 1.25–2.42), respectively. Twenty-four hours after hepatectomy the ammonia-nitrogen content had risen to an average value of 1.28 mg. % and the glutamine-nitrogen to 2.08 mg. %. There was usually a parallelism between the rise in concentration of ammonia and of glutamine in the plasma following complete hepatectomy.

Effects of graded hypoxemia on the pulmonary circulation of the dog: severe hypoxemia. RICHARD GORLIN AND BENJAMIN M. LEWIS (introduced by WALTER T. GOODALE). *Med. Dept., Peter Bent Brigham Hosp. and Harvard Med. School, Boston, Mass.*

Eleven dogs, anesthetized with morphine-chloralose-urethane, were subjected to breathing mixtures containing from 2.5–10% oxygen on 17 occasions. Arterial oxygen saturation varied from 8 to 55% depending on inspired oxygen percentage. Mean pulmonary artery (PA) and left atrial (LA) pressures were measured with indwelling catheters via jugular vein and femoral artery respectively. Integrated cardiac outputs (CO) were measured by the Fick principle, utilizing Benedict-Roth method for O_2 consumption. Pulmonary vascular resistance was calculated: $\text{PVR} = \frac{\text{PA} - \text{LA}}{\text{CO}} \times 1332$ dynes sec. cm.$^{-5}$ Observations were made at intervals varying from 3½ minutes to 8 hours after induction of hypoxia. When arterial oxygen saturation was above 15% and less than 55%, CO increased from 40 to 300% above control values. Oxygen consumption remained unchanged. PA pressure rose moderately; LA pressure remained the same or decreased. PVR remained unchanged or actually decreased, regardless of the time duration of hypoxia. When arterial saturation was less than 15%, LA pressure rose. In 3 observations CO increased and in 6 remained the same or decreased. PA pressure increased uniformly. No changes in PVR were observed. In these experiments arterial saturation was so low that tissue oxygen demands could be met only by increases in blood flow. Under these circumstances, although there was a wide variation in degree and duration of the severe hypoxemia and in the cardiac response to hypoxemia, no alterations in pulmonary vascular resistance were demonstrable.

Kinetics of phosphate transport in erythrocytes. D. R. H. GOURLEY AND J. T. MATSCHINER.* *Dept. of Pharmacology, Univ. of Virginia Med. School, Charlottesville.*

A study of the time-course of the distribution of P^{32} between plasma and the acid-soluble phosphorus compounds of human erythrocytes (*Federation Proc.* 10: 300, 1951) has been completed. The experimental data are consistent with the hypothesis that phosphate enters the erythrocyte by formation of ATP on the cell membrane. The data do not exclude the possibility that inorganic phosphate diffuses into these cells. When the logarithm of the relative specific activity of the plasma inorganic P is plotted against time for the 2-hour period following the addition of P^{32}, the resulting curve may be approximated by two straight lines. This is consistent with the simultaneous occurrence of two processes, one rapid and the other slow, by which the labeled phosphate is removed from human plasma. It is probable that the rapid process (half-time = 10 min.) represents initial diffusion of phosphate into the cells while the slow process (half-time = 300 min.) represents the transport of phosphate into the cells by formation of ATP. In rabbit blood, similar data for the relative specific activity of the plasma inorganic P may be described by a single straight line. The slope of this line is similar to that of the second portion, or slower phase, in human blood (half-time = 200 min.). This suggests that rabbit erythrocytes do not take up phosphate ions both by diffusion and by chemical transport, but only by a chemical process, such as the formation of ATP.

Relationship between total coronary flow, arterial blood pressure and cardiac output. G. R. GRAHAM AND F. WILLIAMS (introduced by L. N. KATZ). *Cardiovascular Dept., Med. Research Inst., Michael Reese Hosp., Chicago, Ill.*

A new method has recently been developed which allows the direct, simultaneous and continuous measuring of total coronary flow, venous return and, thus, cardiac output in the open-chested, but otherwise intact, dog (S. RODBARD, G. R. GRAHAM AND F. WILLIAMS, in press). Essentially this consists of diverting the blood flow from the superior and inferior venae cavae to the left pulmonary artery. The flow through the right pulmonary artery thus carries total coronary

flow only. The sum of the two is the total cardiac output. Utilizing this procedure, data have been obtained on the separate influence of *a*) blood pressure and *b*) cardiac output on total coronary flow. These results will be presented. They indicate that coronary flow varies directly with increases in arterial pressure and cardiac output when these are varied independently, the heart rate remaining constant. This confirms previous work of this laboratory that changes in cardiac output, other factors remaining constant, can alter coronary flow. The control total coronary flow, expressed as a percentage of cardiac output, varies between dogs from 8–20%. In a given dog, when the preparation has functioned for several hours, the percentage of coronary flow gradually increases, although blood pressure and cardiac output may remain relatively unchanged.

Inactivation of pregnant mare's serum gonadotrophin by various species of Lithospermum. R. C. GRAHAM* AND R. L. NOBLE. *Collip Med. Research Lab., Univ. of Western Ontario, London, Canada.*

It has been reported in a previous communication that very small amounts of extracts of the plant lithospermum ruderale are capable of directly inhibiting various gonadotrophins when mixed with them and incubated for 2 hours at 37° prior to injection in immature female rats. A few common weeds and 4 species of Lithospermum (*L. ruderale, L. disticum, L. croceum* and *L. latifolium*) were picked at various seasons. Water extracts of these plants were assayed with pregnant mare's serum gonadotrophin *in vitro* to determine the relative amount of hormone inactivation. In addition, lyophilized extracts of *L. ruderale* and corresponding amounts of a few tannins (Quebracho, Wattle, Chestnut and U. S. P. tannic acid) were assayed in a similar manner. Heating extracts of *L. ruderale* for 10 minutes at 80° C. completely destroyed its antigonadotrophic activity, whereas any activity of the tannins was not diminished. The 4 species of Lithospermum were all effective in causing inactivation of the P.M.S. An unpurified aqueous extract of *L. latifolium* was 80–100 times more active than comparable amounts of other species, 12 μg. total solid inactivating 100 IU (333 μg.) P.M.S. preparation. Seasonal variation affected the amount of activity contained in the roots and tops of the Lithospermum. At the low dose level employed the Lithospermum extracts did not produce any toxic manifestations in the assay rats.

Histological changes in gastric mucosa produced by feeding and the relationship of these to a working hypothesis of growth pattern changes. RHODA GRANT. *Dept. of Clinical Science, Univ. of Illinois College of Medicine, Chicago.*

The gastric fundic and pyloric mucosa during the period of digestion differed histologically from the fasting condition in the following ways: a mucicarmine-stainable secretion covered the surface and filled crypts. It contained epithelial cells or nuclei of these cells, some of which were columnar gastric surface cells. The crypts and lumina in the neck region were widened. The secretion was thicker and more abundant in the pyloric part than it was in the fundic part. Observations were made on dogs, cats and kittens 4½ to 7 hours after feeding meat. From these observations, which confirm a preliminary report on cats (*Canad. M. A. J.* 51: 577, 1944), it is concluded that *1*) cell shedding is a physiological process during meat digestion; *2*) at least for the first 7 hours of the digestion period of meat mucin is a normal component of gastric secretion. Our current working hypothesis that factors which act to decrease the loss of cells, or interfere with the kinetics of the replacement mechanism without reducing the normal mucosal mitotic activity, may alter the growth pattern in the direction of tumor formation will be discussed in the light of these findings.

Latency of response to bacterial pyrogens as affected by previous incubation with blood. RONALD GRANT AND WILLIAM J. WHALEN.* *Dept. of Physiology, Stanford Univ., Stanford, Calif.*

Febrile reactions to bacterial pyrogens involve, an unexplained latent period which is shortened, but not abolished, by large doses. It has been suggested that fever is due to products of general tissue injury, not to a direct action of pyrogens on thermoregulatory centers. Incubation of small amounts of pyrogen (Pyromen, Baxter Labs., derived from *Pseudomonas aeruginosa*) with heparinized autogenous or homologous whole blood before injection may hasten the onset of febrile reactions (inhibition of polypnea, ear vasoconstriction, rise of rectal temperature) without augmenting the fever obtained. No such effects occur with heparinized or citrated plasma although earlier and stronger fevers may occur with other pyrogens in citrated plasma, as shown by LeQuire (*J. Infect. Dis.* 88: 194, 1951). No satisfactory explanation is yet available for either the accelerating or the augmenting effect and conditions for the appearance of both are being studied further. The results lend some support to the notion that an initial peripheral effect is involved in the causation of fever but are taken to indicate that the hypothetical 'injury products' are not produced in important amounts by blood or that the time involved in forming such products is not the main cause of latency.

Comparison of effect of *l*-epinephrine and *l*-norepinephrine on the denervated heart of un-

anesthetized dogs. RUVEN GREENBERG AND CHARLES B. LAMBETH.* *Carter Physiology Lab., Univ. of Texas Med. Branch, Galveston.*

The chronic unanesthetized denervated heart preparation was used to examine for the direct chronotropic effects of *l*-epinephrine and *l*-nor-epinephrine. Contrary to the prevailing concepts: *1*) *l*-nor-epinephrine was a better positive chronotropic agent than *l*-epinephrine by roughly 100%; *2*) there was little or no development of supersensitivity to either compound in the chronic denervated heart.

Cerebral cortical potentials in anoxia. ROBERT G. GRENELL. *Psychiatric Inst., Univ. of Maryland School of Medicine, Baltimore.*

In a series of experiments designed to study the effects of anoxia on the activity of cortical neurons, 2 types of responses to direct electrical stimulation of the suprasylvian gyrus of cats, have been recorded: *1*) the 'dendritic' and 'neuronal' responses recorded from the surface of the hemisphere being stimulated, the stimulating and recording electrodes being approximately 4–5 mm. apart; *2*) the transcallosal response recorded with the stimulating electrodes on one hemisphere and the recording electrode in the same area of the other hemisphere. Records are made of both types of responses while the cats inspire air, 10% oxygen, 7% oxygen and 100% nitrogen through a tracheal cannula. The effects of anoxia on the components of these responses will be discussed. Specific attention will be given to the rates of decay and recovery of the potentials under these various conditions. An attempt will be made to come to some conclusions concerning the relative susceptibility of parts of the neuronal network to anoxia. Preliminary experiments with animals breathing 100% nitrogen have shown the transcallosal response to be much more significantly affected than the ipsilateral—particularly more affected than the 'neuronal' component of the ipsilateral response.

Mechanisms in the bat larynx for production of ultrasonic sounds. DONALD R. GRIFFIN. *Dept. of Zoology, Cornell Univ., Ithaca, N. Y.*

Bats guide their flight by emitting short pulses of ultrasonic sound and listening to the resulting echoes. In *Myotis lucifugus* these pulses have a duration of only 2 msec. (0.002 second), a peak intensity of 10 to 100 dynes/cm.^{2} (95 to 115 db), and a frequency which drops during the single pulse by about one octave (for example from 80 to 40 kc.). In the bat larynx where these pulses of sound are generated the vocal and ventricular folds are replaced by membranes less than 10 micra thick which are stretched like miniature drum-heads over laryngeal ventricles roughly 0.0001 cm.^{3} in volume. Section of the superior laryngeal nerves (which supply the relatively massive crico-thyroid muscles) causes a drop in frequency from the ultrasonic range to an easily audible 'peep' of 8 to 10 kc. After this operation the frequency modulation is almost completely absent. Section of the inferior laryngeals has no noticeable effect on sound production. Direct measurements of the movement of the membranes when subjected to forces perpendicular to their surfaces show that in *Eptesicus fuscus* the crico-thyroid muscles can easily produce a membrane tension of 10 gm. Conventional formulae predict that in this species the stretched membrane would have a fundamental frequency of 45 kc.; and this agrees well with the actual average frequency at the peak of the emitted pulse (40 kc.). The possible role of the laryngeal muscles in the frequency modulation of the pulse will also be discussed. (This research was supported in part by a research contract between the Office of Naval Research and Cornell University.)

Low sodium diet and resin therapy in hypertension. R. S. GRIFFITH,* O. M. HELMER* AND K. G. KOHLSTAEDT. *Lilly Lab. for Clin. Research, General Hosp., Indianapolis, Ind.*

Fifteen patients with essential hypertension were placed on a 200 mg. sodium diet. In addition, a mixture of cation-anion exchange resins was given orally in order to determine, in as short a time as possible, which subjects could be benefited by sodium deprivation. In all cases it was found necessary to administer supplementary potassium orally at the time when the body was maximally conserving sodium. By following this regimen, 8 patients had a drop in blood pressure to normal levels within 2 weeks to 1 month. The blood pressure of 2 patients decreased significantly but not to normal levels. Five patients did not respond to this management. After the blood pressure was stabilized at a normal level the subjects were placed on a 1.5 gm. sodium diet and the resin dosage increased so as to maintain the urine sodium at the same level. No rise in blood pressure occurred while the patients were receiving this amount of sodium. The urine sodium of the patients who failed to respond to therapy could not be reduced to as low levels as the urine sodium of the patients in whom the regimen was successful. In patients who had significant lowering of blood pressure the body weight and the transverse cardiac diameter were decreased, as reported with other forms of salt restriction. In those subjects whose blood pressure was reduced to normal there was evidence of adrenal stimulation, indicated by a drop in absolute eosinophiles and an elevation of plasma renin-substrate concentration. Patients who showed no lowering of blood pressure had neither eosinophile depression nor change in the renin-substrate.

Reversible fibril formation exhibited by a protein in pancreatic extracts. JEROME GROSS (introduced by FRANCIS O. SCHMITT). *Research Labs., Med. Clinic, Massachusetts General Hosp., Boston.*

The author has recently described the presence of characteristically coiled, water-insoluble threads, about 120 A in width, in relatively large numbers in nonfiltered commercial crystalline trypsin, observed with the electron microscope (*Proc. Soc. Exper. Biol. & Med.* 78: 241, 1951). It was also noted that these threads could be produced from sterile, thread-free, crystallized trypsinogen solutions by incubation at pH 7–8.5 at 5° C. It has been found subsequently that relatively large quantities of coiled threads could be obtained incident to production of trypsin from crude wet cake of trypsinogen by a method of Kunitz and Northrup (dissolving in ½–1 volume borate buffer pH 9 and half saturating with $MgSO_4$). Water-washed preparations of such coiled threads in the form of milky suspensions are rapidly turned to water-clear solutions by reducing the pH below 2.5. Electron microscope examination shows a complete absence of threads, coiled or uncoiled. Neutralization to pH 4.5–8.5 produces a thin, opalescent gel, electron microscope examination of which revealed the reappearance of large masses of typical coiled and uncoiled threads. This process is readily reversible. Purified preparations of threads (homogeneous in the electron microscope) have a N = 13.1%, and acid solutions give a typical protein UV absorption curve. The conditions of extraction, fiber formation and reversibility are markedly different from those for insulin described by Waugh. The nature of this protein and its relationship to the pancreatic enzymes will be discussed.

Measurement of cardiac output by a new modification of the Fick principle without gas analysis. J. GROSSMAN, R. E. WESTON AND L. LEITER (introduced by H. HAIMOVICI). *Med. Div., Montefiore Hosp., New York City.*

By means of the Fick principle, any substance with a measurable plasma clearance may be employed to determine the cardiac output. Ideally, the substance should be efficiently removed from the blood in order to produce a large arterio-mixed venous difference, and should be accurately measurable in body fluids. Moreover, if a significant A-V difference is to be attained, the substance must be introduced into the lesser circulation. In these studies, this was accomplished by injecting para-aminohippurate (PAH) or para-acetyl aminohippurate (PACA) into the pulmonary artery via the distal opening of a double lumen catheter by means of a constant perfusion pump. Mixed venous blood was withdrawn from the outflow tract of the right ventricle through the proximal lumen. Following equilibration, urine and arterial and mixed venous blood samples were collected at appropriate intervals. Cardiac output may be expressed as follows from formulae to be derived:

$$\text{Cardiac output} = \frac{UV}{\frac{A - V}{1 - \text{Hct.}}}$$

where A and V are the arterial and mixed venous plasma concentrations respectively, UV is the urinary excretion rate of PACA, and Hct. the hematocrit. When plasma levels (arterial) are constant, rate of injection equals rate of excretion and may replace or serve to check the accuracy of urine collections. In a series of human subjects, cardiac outputs measured simultaneously by the oxygen and PACA methods correlated well.

Nature of effect of the bicarbonate ion on nerve activity. LEONARD GRUMBACH AND HEINRICH H. PROBST (introduced by ABRAHAM M. SHANES). *Dept. of Physiology, Des Moines Still College of Osteopathy and Surgery, Des Moines, Iowa.*

Isolated sciatics of *Rana pipiens*, soaked 1 hour in isotonic $NaHCO_3$ before mounting in a moist chamber perfused with 100% O_2, become almost completely inexcitable after about 1½ hours in the chamber. Change of the gas to 5% CO_2 in O_2 restores excitability almost completely within 8 minutes. The after-potentials in 5% CO_2 are large. This cycle can be repeated many times, the maximal spike heights and after-potentials declining in magnitude with each repetition. The hypothesis that $NaHCO_3$ combines with Ca causing a rapid leakage of K^+ from fibers into the extracellular space and that in 5% CO_2 K^+ is restored to fibers by exchange for H^+ is supported by the following: *1*) Excitability can also be restored to various degrees by washing in isotonic acetic acid (most effective), NaCl, $NaHCO_3$, and mixtures of Na_2CO_3 and $NaHCO_3$ (least effective). *2*) Mixtures of $KHCO_3$ and $NaHCO_3$ accelerate decline of excitability in proportion to K concentration; when K is high, 30% CO_2 is needed to restore excitability. *3*) 100% N_2 accelerates decline slightly and 100% O_2 following N_2 restores excitability within 10 minutes especially if glucose is present; subsequent decline is slower than control. Glucose decelerates decline in both N_2 and O_2. *4*) Pretreatment with high Ca-Ringer's slows subsequent rate of decline of excitability in bicarbonate. Nerves presoaked in isotonic NaCl 12 or more hours are relatively unaffected by $NaHCO_3$ and only slightly restored by CO_2.

Observation of ovulation in the human being via culdoscopy. H. S. GUTERMAN AND M. R. COHEN.* *Dept. of Metabolic and Endocrine Research, Med. Research Inst., Michael Reese Hosp, and Dept. of Obstetrics and Gynecology, Michael Reese Hosp., Chicago, Ill.*

A report will be made of the chance observation of spontaneous ovulation in the human being *in situ*. The appearance and actions of the components participating in the process will be described. The significance of this type of ovulation to problems of reproduction will be discussed.

Phototurbidimetric determination of pancreatic amylase. PAUL GUTH,* S. A. KOMAROV AND HARRY SHAY. *Samuel S. Fels Res. Inst., Temple Univ. School of Medicine, Philadelphia, Pa.*

Waldron's phototurbidimetric (Klett-Summerson colorimeter) determination of pancreatic amylase (*J. Lab. & Clin. Med.* 38: 148, 1951) utilizes the monomolecular constant as measure of enzyme concentration. We found this applicable only over an inconveniently narrow range. With Waldron's technic and our modifications, excellent rectilinear regression [$\log_{10}$ P.N. = $a + b\ (C_0 - C_t)$] obtains over 100-fold (approx. 200–20,000) range of P.N. P.N. = protein nitrogen gamma/100 ml.; C_0 = initial starch concentration = blank = 100%; C_t = undigested starch concentration at end of incubation (read from calibration curve as percentage of C_0). Seven specimens of pancreatic juice from Pavlov-Babkin's fistula dog after standard meal were used in 9 serial dilution experiments. Mixture of 7 parts phosphate buffer (Waldron) and 2 parts M/1 NaCl was used for dilutions and substrate preparation. Modified procedures (*II*, *III*) employed 5 ml. 20% Zippy starch substrate and 5 ml. prewarmed enzyme solution (containing 2 ml. diluted juice). Procedures: *I*) Original Waldron's; *II*) incubation 360 ± 5 sec. at 30°; *III*) incubation 180 ± 5 sec. at 37°. The results are tabulated: r = correlation coefficient; S. D. = standard deviation % error for P. N.; n = number of observations.

	n	a	b	S.D.	r
I	24	1.1038	0.0542	16.88	0.991
II	28	1.2560	0.0520	5.63	0.998
III	15	1.5529	0.0446	9.24	0.998

These experiments demonstrate constancy of amylase/P. in secretion under similar conditions.

Vasomotor waves due to oscillating blood pressure control mechanisms. ARTHUR C. GUYTON. *Dept. of Physiology and Biophysics, University of Mississippi School of Medicine, University.*

In a previous study, pressoreceptor-autonomic oscillation was found to be a probable cause of many if not most of the vasomotor (Traube-Hering) waves ordinarily recorded in blood pressure tracings (*Am. J. Physiol.* 165: 158, 1951). It was predicted that the ischemic reflex receptors of the CNS vasomotor centers, in combination with the autonomic nervous system should also cause blood pressure oscillations under appropriate conditions. This prediction was based on the fact that the ischemic reflex, like the pressoreceptor reflex, causes a negative response, and there is a time delay in the development of this response. When these two conditions are found in a reflex control system, whether or not oscillation will occur depends primarily on the relative sensitiveness of the reflex with respect to the degree of dampening in the system. In the present experiments, the CNS ischemic reflex was made very sensitive by elevation of the cerebrospinal fluid pressure to approximately 120 mm. Hg. Then, the dampening effect of fluid shifts into and out of the circulation presumably was reduced by appropriate quantity of blood removal. Under these conditions, vasomotor waves of more than 100 mm. Hg, with an average period of 23 seconds, occurred in 19 dogs. The oscillating system is explained as follows: CSF pressure causes CNS ischemia and resulting sympathetic excitation; blood pressure rises above CSF pressure, vasomotor center ischemia abates, and sympathetics become less active; blood pressure falls below CSF pressure, vasomotor center ischemia results, and the cycle begins once again.

Effect of pressoreceptor reflex and Cushing reflex on urinary output. ARTHUR C. GUYTON, LEO J. SCANLON* AND GEORGE G. ARMSTRONG.* *Dept. of Physiology and Biophysics, Univ. of Mississippi School of Medicine, University.*

It was reasoned that the inconclusive results from previous studies on the effect of the carotid sinus reflex on kidney output might have been due to the dual action of sympathetic excitation, namely, increased systemic arterial pressure tending to increase glomerular filtration and, secondly, vasoconstriction within the kidney. Therefore, urinary output experiments were performed in 12 dogs, in each of which pressoreceptor or Cushing reflexes were elicited approximately 10 times. In half of these experiments the arterial pressure was kept constant by controlled infusion and withdrawal of blood. When the carotid sinus reflex was elicited without control of blood pressure, kidney output sometimes decreased and sometimes increased, but, when the blood pressure was kept constant, output invariably decreased 2- to 3-fold. When the Cushing reflex was elicited by rapidly elevating CSF pressure to approximately 300 mm. Hg, kidney output decreased 30- to 60-

fold regardless of whether or not the blood pressure remained constant, though the effect was usually slightly greater with controlled blood pressure. Kidney output, as recorded from ureteral catheters, began decreasing within 20 seconds after initiation of each reflex, reached maximum effect in about 70 seconds, and returned to the control level within 2 to 3 minutes after abrogating the reflex. It is suggested that this invariable oliguric response of the kidney to these reflexes may play an important role in the control of blood pressure.

Cerebral vascular resistance, oxygen uptake, jugular venous oxygen saturation and cerebral oxygen tension in patients with essential hypertension before and after a placebo intravenous injection. JOSEPH H. HAFKENSCHIEL, CARL K. FRIEDLAND* AND CHARLES W. CRUMPTON.* *Robinette Fndn., Med. Clinic, Hosp. of Univ. of Pennsylvania, and Dept. of Pharmacology, Med. School, Univ. of Pennsylvania, Philadelphia.*

In order to obtain data for comparison in studies of the effect of depressor and pressor drugs on cerebral hemodynamics, oxygen uptake and cerebral oxygen tension, 10 patients with essential hypertension (4 studied after 88–98% adrenalectomy) were given a placebo physiological saline injection. Measurements were repeated using the nitrous oxide technique 60 minutes later. Mean changes for the unoperated group were: mean arterial pressure (MABP) 152–144 mm. Hg (±7), cerebral blood flow (CBF) 65–60 cc/100 gm/min. (±12), cerebral vascular resistance (CVR) 2.8–2.5 (±1.1), cerebral oxygen uptake ($CMRO_2$) 3.8–3.4 cc/100 gm/min. (± 1.7), venous oxygen content (VO_2) 11.6–11.4 vol. % (± 0.8), venous oxygen saturation (V sat.) 64–63% (±4), venous hydrogen ion conc. (V pH) 7.32–7.35 (±0.06), calculated venous oxygen tension (VpO_2) 37–36 mm. Hg (±2), the latter assumed to represent cerebral oxygen tension. The operated patients were studied 3–15 months after surgical treatment. Mean values obtained in this group were: MABP 115–117 (±6), CBF 63–60 (±22), CVR 2.0–2.1 (±0.8), $CMRO_2$ 4.1 unchanged (±1.8), VO_2 10.7–10.4 (±0.4), V sat. 60–59 (±3), V pH 7.29–7.31 (±0.03), VpO_2 35–33 (±3). There were no significant differences from the initial values and the test response of the 2 groups was essentially the same. Cerebral oxygen tension was no lower than the range 27–55 mm. Hg. (This study was supported by research grants from the National Heart Institute (H-361), U. S. Public Health Service, Sandoz Pharmaceuticals, Ciba Pharmaceutical Products, Inc., Eli Lilly and Company and the Squibb Institute for Medical Research.)

Effects of ethyl urethane and sodium azide on growth and respiration in *Chilomonas paramecium* RICHARD J. HAHN (introduced by CHARLES G. WILBER). *Univ. of Nebraska, Lincoln.*

The effects of ethyl urethane in concentrations of $1–10^{-12}$ M and sodium azide in concentrations of $1–10^{-10}$ M were observed on growth and respiration in *Chilomonas paramecium*. One molar ethyl urethane resulted in cytolysis of the organisms within 48 hours and inhibited respiration by 69%. 10^{-2} M ethyl urethane inhibited growth by 34% and respiration by 19%. No significant differences were noted in lower concentrations. One molar concentration of sodium azide resulted in death of the organisms within 2 hours. Sodium azide in a concentration of 10^{-2} M caused complete inhibition of growth and a 67% inhibition of respiration while 10^{-3} M completely inhibited growth and inhibited respiration by only 27%. A 10^{-4} M concentration of sodium azide inhibited growth by 27% and respiration by 8%. All other concentrations of sodium azide resulted in no significant changes.

Diurnal variations in amount of electroencephalographic paroxysmal discharge and diurnal eosinophil rhythm of epileptics on days with clinical seizures. F. HALBERG, R. ENGEL, E. HALBERG AND R. J. GULLY (introduced by M. B. VISSCHER). *State Hosp., Cambridge, Minn., and Dept. of Physiology, Univ. of Minnesota.*

Electroencephalographic tracings of 4-minute duration were obtained from epileptics at 90-minute intervals over 24-hour periods. Venous blood for eosinophil counts was withdrawn upon completion of these tracings. The exact time of the incidence of fits, if any occurred during the investigations, was recorded. The diurnal changes in the amount of paroxysmal discharge were computed by expressing for each 4-minute record the duration of paroxysmal discharge as percentage of the total duration, by getting the mean of the 16 values thus obtained during a 24-hour period, and by converting the duration of each paroxysmal discharge into a percentage of the mean value of the duration of all paroxysmal discharges for that day. The variations of the eosinophil counts were expressed as relative eosinophil levels. The records from days with clinical seizures provide data for studying the variations in the amount of paroxysmal discharge and in the numbers of eosinophils —in relation to fits. The paroxysmal discharge at or around the time of fits was found to be well above the diurnal mean. On the other hand most of the clinical seizures noted occurred at eosinophil levels below the diurnal mean. The inference is drawn that the occurrence of fits at or close to the times of the diurnal minima in eosinophils may indicate a precipitating influence of eosinopenic corticoids in epilepsy.

Comparison of eosinophil responses in human subjects during flights in aircraft, decompression to 40,000 ft., and exposure to cold. HENRY B. HALE AND JOHN E. KEATOR (introduced by HARRY F. ADLER). *USAF School of Aviation Medicine, Randolph Field, Tex.*

A fall in circulating eosinophils is considered an index of 11-oxysteroid output from the adrenal cortex and therefore a measure of the degree of 'stress' imposed by a test situation. The average change in eosinophil level for a group of 100 medical officers (average age, 30) was determined in several 'presumably stressful' situations. Results from similar tests on other groups (flying personnel and altitude chamber technicians) were also obtained. The following test situations were studied: *1*) a 2-hour flight in a C-47 type aircraft which included a 30-min. period at 15,000 ft. without supplemental oxygen; *2*) decompression in an altitude chamber to 40,000 ft. using oxygen equipment only after reaching 18,000 ft.; *3*) decompression to 40,000 ft. at −65° F. using oxygen equipment and electrically-heated clothing; and *4*) a 2-hour exposure to −40° F. at ground level with a 5 mph wind, the subjects wearing standard Air Force intermediate clothing assembly for cold protection. For 72 subjects it was possible to secure control determinations on days when no test situations were conducted. As an example of the results, the average fall in eosinophils for a group of 46 subjects over a 3-hour period varied as follows: 8% on the control day, 18% for the C-47 flight, 23% in response to cold at ground level, and 40% during decompression to 40,000 ft. Other results follow this pattern and will be presented and discussed.

Fundamental relationship between tissue oxygen tension and radiosensitivity. B. VINCENT HALL (introduced by ROBERT E. JOHNSON). *Biomed. Div., Argonne Natl. Lab., Chicago, Ill., and Dept. of Zoology, Univ. of Illinois, Urbana.*

Radiosensitivity was determined by measuring the growth and viability in host mice of transplanted tumor fragments (anaplastic carcinoma) exposed to X-radiation *in vitro* under standardized conditions. (Radiation factors: 200 kv; 8 mm Al; dose rate, 1030 r/min.; t.d. 12.7 cm.) Treatment with 0.01 M sodium cyanide and chilling to 0°C. both greatly increased the radiosensitivity of tumor fragments irradiated in atmospheric oxygen. In nitrogen treatment with cyanide and cold did not affect radiosensitivity. Radiosensitivity of tumor fragments simultaneously chilled (0°C.) and treated with 0.01 M NaCN was not significantly different from that found after treatment with either of these agents alone. Treatment with cold, cyanide or nitrogen did not influence the growth or viability of non-irradiated control tumor fragments. Neither did such treatment when it followed irradiation. Exposure of tumor fragments to 0.01 M NH_2OH and 0.01 M NaN_3 had little effects upon their radiosensitivity. Measurements of oxygen consumption, by the Warburg method, showed HCN to be much more effective than NH_2OH or NaN_3 in depressing oxidative metabolism of tumor slices *in vitro*. Additional experiments showed that the radiosensitivity of tumor fragments of appropriate dimensions in atmospheric oxygen is an inverse function of their size. The variations in radiosensitivity reported here for tumor fragments, and by many other workers reported elsewhere, produced by cold, anoxia, cyanide, and other agents can be fully correlated with changes in tissue oxygen tension as calculated by the Krogh formula, relating the penetration of oxygen into tissues, supplied with oxygen solely by diffusion, to the environmental oxygen tension, a diffusion constant, and the metabolic rate.

Cortisone and hypertensive hyalinosis in the rat. C. E. HALL AND O. HALL.* *Carter Physiology Lab., Univ. of Texas Med. Branch, Galveston.*

The spontaneous development of hypertension, renal arteriosclerosis and glomerulosclerosis, myocarditis, arthritis and periarteritis nodosa in parabiotic rats has been described (Hall and Hall). Rats which had developed a moderate to severe hypertension as a result of being placed in parabiosis were studied. In each case the hypertensive partner of a pair was treated with 1–3 mg/day of cortisone acetate s.c. This almost invariably resulted in a decline of blood pressure to normal levels. The period necessary to achieve normalization was from 1–6 weeks; the animals which had the greatest degree of hypertension and which had been hypertensive longer prior to treatment were slowest in recovery. Failure to reduce hypertension was encountered in only one case, in which a severe degree of periarteritis had developed prior to treatment. Cardiovascular-renal lesions were minimal in all except this latter case. Healed periarteritic lesions were observed in some of the rats in which the blood pressure had returned to normal following cortisone treatment. (Supported by a grant from the American Heart Association.)

Regulation of breathing of man at altitude. F. G. HALL. *Dept. of Physiology and Pharmacology, Duke Univ. School of Medicine, Durham, N. C.*

When man breathes ambient air at high altitudes, an hypoxic stimulus causes a rapid increase in pulmonary ventilation. The full effect of this hypoxic stimulus is counteracted, however, by concomitant hypocapnia. With the addition of carbon dioxide to inspired air a more effective response to the hypoxic stimulus is manifested. It is concluded that while hypoxia and hypercapnia are separate stimulating factors and addi-

tive in their effects, the actual regulation of respiration at altitude depends upon the manner of their interaction.

Independence of diffuse thalamo-cortical projection system shown by specific nuclear destruction. JOHN HANBERY* AND HERBERT H. JASPER. *Montreal Neurological Inst. and McGill Univ., Montreal, Canada.*

The pathways of the diffuse thalamo-cortical projection system to the cortex were investigated in cats anesthetized with Nembutal. Recruiting responses over widespread areas of cortex, including sensory areas, were obtained from certain well localized zones in the thalamic reticular system, which includes the centre median and intra-laminar nuclei as well as n. ventralis medialis and anterior of the thalamus, the latter nuclei giving short latency diffuse cortical projections as compared to more restricted longer latency recruiting responses from centre median. Recruiting responses were tested before and after electrolytic destruction of various specific relay and association nuclei of the thalamus. After complete destruction of the geniculate bodies it was possible to set up very good recruiting responses in visual and auditory cortex even though evoked potentials to sound and light stimuli could no longer be induced. Recruiting responses continued in the post-cruciate sensory cortex after complete destruction of the sensory relay nucleus VPL in the thalamus. Likewise, complete destruction of n. medialis dorsalis did not prevent setting up good recruiting responses in the frontal cortex, and destruction of n. ventralis lateralis did not affect recruiting responses in the motor cortex. Very large destruction of practically all of the lateralis posterior-pulvinar complex also failed to prevent recruiting responses from the parietal cortex of the mid-suprasylvian gyrus. A relatively small destruction of the pathways of the diffuse projection system in the rostral thalamus, will abolish generalized recruiting responses even though specific projection nuclei are not affected. It is concluded that the diffuse thalamo-cortical projection system does not act via specific thalamic nuclei but has independent projections to the same cortical areas (including sensory) as are supplied by more concentrated projections from specific thalamic nuclei.

Trauma and creatinine excretion. JAMES D. HARDY. *Harrison Dept. of Surgical Research and Surgical Services of Univ. Hosp., Schools of Medicine, Univ. of Pennsylvania, Philadelphia.*

This report presents one segment of a more comprehensive clinical investigation of metabolic alterations following major operations. The daily urinary excretion of creatinine (preformed) was initially measured as a check on the completeness of the 24-hour urine collections, but as data accumulated it was perceived that the excretion of creatinine was consistently elevated following surgical trauma. The average increase was approximately 40%, and the peak usually occurred during the second 24-hour period following that in which the operation was performed. The most marked elevations occurred in those patients who also had markedly febrile postoperative courses, and the relationship between fever and creatinine excretion was observed to be a close one. However, there were exceptions in whom there was a pronounced postoperative rise in creatinine excretion accompanied by only a mild elevation in temperature. Thus, the increased creatinine excretion which accompanies fever is not necessarily a result of the fever; the two may be associated but independent segments of the alarm response. The increase in creatinine excretion was paralleled by an increase in nitrogen excretion and in adrenocortical activity. The serum creatinine level usually rose slightly or remained constant. The observed increase in creatinine excretion has practical implications. If creatinine excretion can be considered to reflect the magnitude of the metabolic activity of the muscle mass, the percentage increase in creatinine excretion which follows a particular operation may prove useful in estimating the impact of this operation upon the metabolism of the patient.

Relationships between fever and sweating. JAMES D. HARDY. *Harrison Dept. of Surgical Research, and Surgical Services of Univ. Hosp., Schools of Medicine, Univ. of Pennsylvania, Philadelphia.*

The increase in body temperature which frequently follows the sterile trauma of simple herinorrhaphy or fracture of a long bone has long puzzled surgeons. Efforts to prove the presence of infection in many of these patients have failed so consistently as to leave little doubt of the presence of some mechanism other than that of 'pyrogenic foreign body reaction.' There would appear to be 3 possible ways in which body temperature can be increased: increased heat production, decreased heat loss or a combination of both. In collecting sweat specimens in a rubber glove for the analysis of electrolyte concentrations, we have found it more difficult to obtain sweat in the early postoperative period than it was preoperatively. In 6 of 10 patients studied no sweat specimen was obtained on the second postoperative day; all subjects sweated preoperatively. The greatest difficulty in obtaining sweat postoperatively tended to occur on days when the urinary creatinine excretion was increased; this correlation was slightly superior to that existing between the ability to sweat and oral temperature. These findings suggest that fever following 'sterile'

trauma may be an integral part of the alarm reaction. It is further suggested that postoperative fever may be maintained in part by the curtailment of heat loss through a central control of sweating. Unless fever rises to a point which threatens the integrity of the vital centers, it perhaps should be viewed as a beneficial segment of the alarm response.

Thresholds for pain and reflex contraction as measured by the thermal radiation method. JAMES DANIEL HARDY. *Dept. of Physiology, Cornell Univ. Med. College, New York City.*

Pain thresholds were measured on the forehead of 3 trained subjects by observing, for different durations of exposure (0.5–90 sec.), the intensity of radiation which would just evoke a sensation of pricking pain. The threshold reflex contraction of flexors of the lower extremities in a paraplegic individual (section at T9–T10) was measured by noting the lowest intensity of radiation evoking a contraction when the dorsum of the foot was stimulated for varying lengths of time. The reflex contraction of the musculus cutaneous maximus of the guinea pig, and the tail flick of the rat, were similarly studied after exposing the depilated skin of the back to thermal radiation. Skin temperatures were measured with a radiometer prior to making threshold measurements. Results of these measurements show that for a particular exposure time the intensity of thermal radiation evoking threshold pain and threshold reflex response is the same. Also, the calculated skin temperature at the time of the threshold pain and threshold contraction was approximately the same, i.e. 44°-45°C., the thermal tissue injury threshold, for all durations of exposure. It is inferred from these observations that *a*) pain and certain reflex responses have the same threshold level as regards noxious stimulation, and *b*) noxious stimulation at least in these instances and possibly in general is closely associated with tissue injury.

Suppressor effects of magnesium salts upon ectopic ventricular tachycardia accompanying myocardial infarction. A SIDNEY HARRIS, ANTONIA ESTANDIA,* HERBERT T. SMITH,* ROY W. OLSEN* AND REX F. TILLOTTSON.* *Baylor Univ. College of Medicine, Houston, Tex.*

Dogs were prepared by 2-stage aseptic surgical occlusion of the anterior descending artery, using pentobarbital sodium anesthesia. During the first and second postocclusion days, without anesthesia and while ventricular tachycardia of relatively stable frequency existed the effects of intravenous $MgSO_4$ and $MgCl_2$ upon the ectopic activity were tested. Repeated injections were made at intervals averaging about 30 minutes until ectopic activity was well controlled or toxic manifestations interfered with further administration. In 4 of 12 dogs with rapid ventricular tachycardias $MgSO_4 \cdot 7H_2O$ in total amounts of 480–860 mg/kg. (4–7 mEq. magnesium/kg.) reduced the ectopic rate to 0 for a brief period and maintained it at less than half of the pre-treatment control rate for 4 hours or longer without observed toxic reactions except vomiting (arbitrary criterion of therapeutic success). $MgCl_2 \cdot 6H_2O$ in total dosage of 400–600 mg/kg. (4–6 mEq. magnesium/kg., approximately) produced successful results (above criterion) in 7 of 12 animals. Deaths during testing occurred in 2 of 12 animals with $MgSO_4$ and 3 of 12 with $MgCl_2$. In another series, pentobarbital sodium prior to testing significantly increased the danger of death with both magnesium salts. Weak hypotensive animals were hypersusceptible to magnesium depression. $MgCl_2$ may be somewhat more effective than $MgSO_4$, relative to toxicity limitations, for the suppression of ectopic impulses in myocardial infarction.

Skin resistance changes following stimulation of hypothalamus. FRANK HARRISON AND S. R. BRUESCH. *Div. of Anatomy, Med. Units Div., Univ. of Tennessee, Memphis.*

The hypothalamus of 26 cats and 2 monkeys under chloralose-urethane anesthesia was stimulated electrically and the skin resistance changes were recorded. The stimulus was for 1 second at 120 cycles, 2 volts, and sine wave form. By means of an electrode held in a stereotaxic instrument discrete regions of the hypothalamus and adjacent areas were activated. Skin resistance changes were recorded from the footpads with clip electrodes through saline-soaked gauze squares and connected to a Wheatstone bridge and Brush ink writing oscillograph. Stimulation of the hypothalamus results in a decrease in skin resistance of the footpads. Stimulation in the region of the tuber cinereum, 2 to 4 mm. lateral to the midline, and midway dorso-ventrally within the hypothalamus, yields a maximal response, which in its fully developed form consists of 3 successive decreases in resistance. The first decrease begins at 0.75 to 2.0 seconds, the second at 2.0 to 3.5 seconds, and the third at 20 to 40 seconds after the beginning of the stimulus. The response is usually obtained in all four limbs upon unilateral stimulation, although the areas from which bilateral fore-limb responses may be obtained is slightly larger than that for the hind-limbs. Responses were produced bilaterally upon stimulation as far caudad as the obex of the medulla. Hind-limb responses lagged behind the fore-limb responses by 0.2 to 0.4 second.

Effects of electric current on responses of single photoreceptor units in the eye of *Limulus*. H. K. HARTLINE, N. A. COULTER, JR.* AND H. G. WAGNER.* *Jenkins Labs. of Biophysics, Johns*

Hopkins Univ., Baltimore, and Naval Med. Research Inst., Bethesda, Md.

Several milliamperes of direct current passed through the eye of *Limulus* caused the discharge of trains of nerve impulses in the optic nerve fibers during the flow of current when the corneal surface was cathodal and after its cessation when the cornea was anodal. With cornea cathodal the discharge in a single fiber began at a high frequency and 'adapted' to a lower level, resembling the response to illumination of the photoreceptor unit supplying the fiber. The frequency increased linearly with the logarithm of the current strength. With cornea anodal, the discharge at 'break' resembled responses to darkening of 'off' elements in other visual systems; the frequency and duration of the discharge were greater the stronger the foregoing current. Threshold to light flashes was increased and frequency of discharge in response to steady suprathreshold illumination was decreased during current flow with cornea anodal; the reverse was true during reversed current flow, even at current strengths too weak to elicit discharges by themselves. After prolonged passage of supraliminal current with cornea cathodal sensitivity was reduced, but was recovered rapidly (in 2 minutes) whether tested by light or electric current. After prolonged illumination at an equivalent stimulating level sensitivity was reduced, and recovery was rapid when tested electrically, but very slow (40 minutes) when tested by light.

Survival of human blood platelets *in vitro*. ROBERT C. HARTMANN AND C. LOCKARD CONLEY (introduced by DAVID GROB). *Dept. of Medicine, Johns Hopkins Univ. and Hosp., Baltimore, Md.*

The survival of human platelets under various conditions was studied by a semi-quantitative measurement of the ability of platelets to produce clot retraction. Observations were made on plasma containing varying concentrations of platelets after the other formed elements of the blood had been removed by differential centrifugation. Platelet preparations were stored at 2°C. for as long as 35 days. In some instances excellent clot retraction occurred even after two weeks storage although there was a progressive decrease in the number of platelets. When platelet preparations were stored at 37°C., clot retraction was good after 48 hours but no longer occurred after the third day. There was no difference in platelet survival whether the preparations were stored in glass or in silicone-treated tubes. When platelet preparations were frozen and thawed, retraction no longer occurred. However, when platelets were frozen in 15% glycerol solution, there was a decrease in the number of platelets, but those remaining were capable of producing retraction. The intensive irradiation of platelet preparations with roentgen rays had no appreciable effect upon retractile function. No difference in clot retraction was noted with platelets obtained from blood of the four major blood groups. Admixture of platelet preparations with incompatible plasma did not cause agglutination of platelets or impairment of clot retraction. Plasma from patients with thrombocytopenic purpura had no effect on normal platelets *in vitro*. Platelets from a patient with thrombocytopathic purpura were defective in clot retractile function although adequate in number.

Effect of oxygen on utilization of ATP for luminescence. J. WOODLAND HASTINGS (introduced by W. D. MCELROY). *McCollum-Pratt Inst., Johns Hopkins Univ., Baltimore, Md.*

Studies by McElroy and associates have shown that light emission in firefly extracts depends upon luciferin, luciferase, Mg^{++}, adenosine triphosphate, and oxygen. When the reactants are mixed in air, the light intensity decays to a low level in about 1 minute. Although this decay is due largely to ATP immobilization, only a negligible quantity is split. It has been proposed that ATP is complexed in a way which makes it unavailable for light production. If the reactants are deaerated and mixed anaerobically, no luminescence appears. Subsequent admission of air results in a bright, but rapidly decaying, flash. The intensity and rate of decay of the flash depend upon the time of anaerobiosis in a way which indicates that the immobilization of ATP occurs in the absence of oxygen. The reaction mixture may be repeatedly deaerated and upon each addition of air there is observed a similar, rapidly decaying flash of luminescence. This indicates that very low oxygen concentrations bring about the accumulation of some key intermediate, probably involving ATP, which is rapidly utilized for light production in the presence of oxygen. By running the reaction at intermediate oxygen concentrations, a differential sensitivity to oxygen of the initial bright luminescence and the basal luminescence may be shown. This suggests that more than one reaction is affected by oxygen concentration.

Effects of preliminary renal ischemia on experimental renal hypertension. EDWARD W. HAWTHORNE* AND GEORGE E. WAKERLIN. *Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.*

The effects of preliminary renal ischemia, produced by bilateral renal artery occlusion for 2, 4, 4½, 5, and 6 hours, on the development of experimental renal hypertension have been studied. Dogs survived a temporary period of renal artery occlusion of as long as 4 hours with regularity. The degree of damage induced by 2, 4, 4½, 5, and 6 hours was extremely variable as determined by renal function studies, including renal clearance

and saturation techniques, and pathologic studies. Dogs with mild renal damage showed the same rise of mean femoral arterial pressure in the first 3 months following bilateral renal artery constriction as did normal control dogs. Dogs with severe renal damage showed slight to no rise of mean femoral arterial pressure for 1–6 months following bilateral renal artery constriction. After this period of relatively normal pressure, usually an abrupt rise in pressure to definitely hypertensive levels occurred. Renal function studies in such dogs suggested that an inverse correlation existed between the ratio of the effective renal plasma flow (C_{PAH}) to the maximal tubular excretory capacity for sodium *p*-aminohippurate (Tm_{PAH}) and the mean femoral arterial pressure following bilateral renal artery constriction.

Fibers of surface-spread actomyosin as models of muscle contraction. TERU HAYASHI AND RAJA ROSENBLUETH.* *Dept. of Zoology, Columbia Univ., New York City.*

Actomyosin fibers, formed by surface-spreading the protein at an air-water interface and subsequent compression, have been demonstrated to have ATP-ase activity and also the capacity for contractility. Activated by ATP, they have been shown capable of lifting a load in isotonic contraction, i.e. to perform mechanical work, thus demonstrating that in the method of fiber formation, one of the requisites of a muscle model, that of structural continuity, has been obtained. The contractions of these fibers are reversible, that is, under isotonic conditions, the fibers are capable of shortening, subsequent elongation, and shortening again in a repeated cycle of events. The condition for shortening is ATP with low KCl concentration in the medium; that for elongation is high KCl concentration. It may be demonstrated that the glycerinated psoas, treated with pyrophosphate, is also capable of repeated contractions and relaxations under similar conditions. Further experiments give evidence that in the actomyosin fibers, the elongation is a passive process, and that both the ATP-ase activity and the contractility are inhibited to the same degree by the same concentrations of Cu ions. The results are discussed in terms of possible relation to the structure of muscle and the mechanism of contraction. It is emphasized that in making a comparison of the behavior of such protein threads and intact or near-intact muscle, the basis for such comparisons be carefully selected, with regard to the differences in such a simplified protein system and the structural and functional complexity of muscle.

Effect of ACTH and cortisone on antibody synthesis and rate of disappearance of antigen. SHELDON P. HAYES* AND THOMAS F. DOUGHERTY. *Dept. of Anatomy, Univ. of Utah College of Medicine, Salt Lake City.*

Administration of graded amounts of ACTH or cortisone when given daily to mice which had received single intravenous injections of graded amounts of horse serum suppressed the synthesis of antibody. The extent of suppression depended upon the amount of antigen given, the time elapsing after the antigen administration and the amount of hormone administered. The greater the amount of antigen the more hormone was required to suppress the formation of antibodies. Although ACTH and cortisone decreased the quantity of antibody formed, the administration of these hormones did not alter the time of appearance or shorten the length of time antibodies circulated once they were formed. Cessation of treatment with either of these hormones at 9, 11 or 17 days after administration of antigen was followed by a prompt resumption of antibody synthesis so that antibody titers equivalent to those of non-hormone treated immunized control animals were found within 48 hours. These results suggested that antigen persisted in the tissues during the period of inhibition of antibody synthesis. This hypothesis was tested by a technic of reverse anaphylaxis. Graded amounts of sera containing anti-horse serum antibodies were given to the hormone treated animals 9 and 17 days after administration of horse serum and beginning hormone treatment. The results demonstrated that ACTH and cortisone produced a more rapid disappearance of antigen than that found in immunized nonhormone treated animals since more antibody was required to produce fatal anaphylaxis in the hormone treated animals. (Supported by a grant from Research Division, Armour and Company.)

Plasma renin concentration of patients treated with the artificial kidney. FLORENCE W. HAYNES, JOHN P. MERRILL* AND WALTER T. GOODALE. *Med. Dept., Peter Bent Brigham Hosp. and Harvard Med. School, Boston, Mass.*

Since a rise in arterial blood pressure is often observed during treatment with the artificial kidney, the presence of renin in the blood was estimated in a series of patients before and after dialysis. Renin was determined in 7 cc. of arterial plasma by incubation with hypertensinogen and bioassay of the hypertensin formed. The hypertensinogen level before dialysis, by similar assay, was normal in most patients. Four patients showed significant amounts of renin (0.6–1.5 CU/10 cc. plasma), 3 doubtful amounts, and 5 none. The amounts of renin before and after dialysis varied slightly but demonstrated no consistent changes. In 9 patients, the mean blood pressure increased during dialysis (9–69 mm. Hg), but the greatest increases were not found in patients with the most

renin. Thus, evidence is against the rise in blood pressure being due to an excess of renin. Of the 4 patients who had significant amounts of renin, all had uremia, 2 had hypertension (1 developed in 7 days after carbon tetrachloride poisoning), and 1 had cortical necrosis. Two died within a few days and 2 recovered. Of the 4 patients of the series with a history of acute oliguria (4–6 days), 3 had positive and 1 a doubtfully positive renin, suggesting an increase in plasma renin shortly after the onset of acute renal damage. The amount of renin showed no obvious correlation with the cardiac output, serum protein, hematocrit, blood urea nitrogen or body temperature.

Observations on the oxygen content of sternal bone marrow with reference to polycythemic states. HANS H. HECHT AND ARTHUR J. SAMUELS (introduced by VIRGINIA D. DAVENPORT). *Dept. of Medicine, Univ. of Utah College of Medicine, Salt Lake City.*

In 40 subjects the oxygen content of aspirated sternal bone marrow blood was determined during rest and following mild exercise. The values were compared with those obtained simultaneously from arterial and mixed venous blood. The subjects examined were divided into several groups comprising normal volunteers (8), patients with 'low output heart failure' (8), chronic obstructive emphysema (3), cor pulmonale with (11) and without polycythemia (7). Three subjects suffered from polycythemia vera. In all instances bone marrow oxygen content and oxygen tension closely resembled the values obtained simultaneously from mixed venous blood by catheterization. Bone marrow oxygen content was lower than normal in all groups with heart disease and reached excessively low values in subjects with cor pulmonale in whom polycythemia had been corrected by bleeding. Arterial-bone marrow oxygen difference measured 2.5 vol. % for normal subjects, 5.7 vol. % for patients of the 'low output failure' group. Intermediate values were obtained in the other groups and varied conversely with cardiac output at rest and exercise. The subjects with polycythemia vera showed no abnormal findings except for the expected increase in oxygen capacity and oxygen content. The data suggest *1*) that aspirated bone marrow blood may be considered venous blood when its gas contents are examined, *2*) that the onset of polycythemia secondary to lung disease occurs at arterial levels of approximately 35 mm. Hg pO_2 and approximately 25 mm. Hg pO_2 bone marrow-venous-oxygentension, *3*) that a state of extremely low oxygentension presumably resembling the conditions that led to the development of polycythemia is uncovered in subjects in whom polycythemia is corrected by bleeding and *4*) that polycythemia vera shows no defect in oxygenation of blood and bone marrow.

Size of retinal arterioles as related to age and blood pressure. AUSTIN HENSCHEL AND ANCEL KEYS. *Lab. of Physiological Hygiene, Univ. of Minnesota, Minneapolis.*

The diameter of the retinal arterioles was related to age and blood pressure in 396 normotensive and asymptomatic hypertensive subjects 18–65 years of age. The retinae were photographed in black and white with a Zeiss fundus camera and the blood vessels measured from enlarged projections of the negatives. Measurements were made at 1-disc diameter distance from the edge of the optic disc. In normotensives the mean arteriolar diameter was 99.6 and 93.2 microns in the 20 and 50 years of age groups, respectively. The difference between the 2 age groups was greater in those with blood pressures of 140/90 or above (98.9 and 86.7 microns for the young and older hypertensives, respectively). In both instances the arterioles were statistically significantly smaller in the older group. Within the older age group, the hypertensives had statistically significantly smaller arterioles than did the normotensives (93.2 and 86.7 microns for the normotensive and hypertensive groups). The data indicate a significant progressive narrowing of the retinal arterioles with increasing age and with increasing blood pressure even in asymptomatic subjects who show no evidence of retinal changes by the usual qualitative ophthalomoscopic procedures.

Proposed method for assay of thyroid preparations. ELIZABETH A. HERROLD, ROY K. RINEHART AND HAROLD E. COOMBS (introduced by JAMES H. LEATHEM). *Research Lab., Ciba Pharmaceutical Products, Inc., Summit, N. J.*

The goiter-inducing effect of thiouracil can be counteracted with thyroxin; this suggested a method for the assay of thyroid powder. Incorporation of 0.1% thiouracil in a commercial diet for 14 days resulted in a 250% increase in the thyroid weights of young, female rats (Wistar strain). Concomitant inclusion of thyroid powder at concentrations of 0.0025–0.02% of the diet with increments of 0.0025% permitted estimation of the activity of the preparation. Plotting dose of thyroid powder against the logarithm of thyroid weight mg. % body weight yielded a straight line between concentration levels of 0.005% and 0.015%. Approximately 0.01% thyroid induced a 50% inhibition of goiter. Responses of thyroid weight became significant both from each other and from those of animals receiving only thiouracil when concentration differences of 0.005% thyroid powder were compared. The biological activity of tissues taken from animals or from human beings

treated with various thyroid and antithyroid preparations can be measured.

Changes in plasma P.B.I. in response to stressful life experiences. B. S. HETZEL, D. S. DE LA HABA AND L. E. HINKLE, JR. (introduced by H. G. WOLFF). *Depts. of Medicine and Psychiatry, New York Hosp.-Cornell Med. Center, New York City.*

Because clinical evidence has suggested that disturbances in thyroid function occur in response to stressful life experiences, experimental studies have been designed to explore this possibility. The protein bound iodine (P.B.I.) concentration in the blood plasma was selected as an indicator of thyroid function and the alkaline ash method of Barker was used. Preliminary studies have shown that a deviation of greater than ± 0.6 *g* was significant. In an experimental setting euthyroid and hyperthyroid subjects were studied before, during and after stressful interviews dealing with topics known to be of threatening significance in their lives. Frequent determinations of P.B.I. were made during the course of a 3- to 4-hour period of observation. Hemoglobin estimations and eosinophile counts were also carried out at the same time. When it was possible to maintain the subject in a relatively neutral state, P.B.I. did not vary significantly. On the other hand, in association with reactions to stress 2 divergent patterns of change were noted. In one the P.B.I. rose by as much as 20% in 2 hours associated with a drop in circulating eosinophiles; in the other, the P.B.I. fell by as much as 15% in 2 hours without a drop in circulating eosinophiles. Variations in hemoglobin concentration occurred but did not appear related to these other changes. The pertinence of these changes to thyroid disease has yet to be ascertained.

Distribution, excretion and metabolism of the nitrate ion administered intravenously as $NaNO_3$. EDWIN P. HIATT AND INEZ GREENE. *Dept. of Physiology, Univ. of North Carolina School of Medicine, Chapel Hill.*

It has long been known that nitrate salts are diuretic and promote the excretion of chloride. as much as two thirds of the total body chloride has been displaced by continued intravenous administration of $NaNO_3$ to dogs. (HIATT. *Am. J. Physiol.* 129: 597, 1940). We are reinvestigating the behaviour of the nitrate ion in the body using a new analytical method involving the enzymatic reduction of nitrate to nitrite, which is then diazotized and determined colorimetrically. The volume of distribution of nitrate is apparently about the same as that of chloride. This ion is excreted via the same renal mechanisms as chloride (i.e. filtration and reabsorption) but reabsorption is much less efficient for nitrate so that it is gradually eliminated from the body fluids. However, so long as nitrate is present, the excretion of chloride is enhanced. During the elimination of the nitrate more sodium is excreted than is injected with the nitrate. Consequently the net effect of the intravenous administration of this salt is an extracellular dehydration. The conversion of nitrate to nitrite in the body after intravenous administration is negligible, at least in short term experiments. About 90% of an administered dose of sodium nitrate can be recovered in the urine.

Hyperpnea following release of arterial tourniquets. JOHN B. HICKAM. *Duke Hosp., Durham, N. C.*

The present report deals with an examination of the stimuli responsible for the increase in ventilation volume which usually follows resumption of circulation through a large muscle mass which has been made acutely ischemic. Arterial tourniquets are applied to both thighs of recumbent subjects, and the legs are exercised until the onset of ischemic discomfort. After a minute of rest the tourniquets are released. Ventilation is recorded continuously, and arterial blood samples are taken at intervals throughout the procedure. Discomfort is not great and disappears rapidly within a few seconds after release of the tourniquets. Major ventilation changes during or immediately after manipulation of the tourniquets are rare. Fifteen to 40 seconds after release of the tourniquets most subjects show an abrupt increase in ventilation, amounting to about 30% of the resting level and lasting 30–60 seconds. The ventilation change is reduced or abolished when the subject breathes oxygen and is intensified by breathing 15% oxygen. During the ventilation increase there is a transient elevation of arterial blood pCO_2 of 5–10 mm. Hg and a fall in arterial pH, but ventilation changes are not well correlated with these blood changes. Simultaneously, there is a small drop in arterial % oxygen saturation, unless the subject is breathing oxygen. The finding that this ventilation change is oxygen-sensitive but yet associated with changes in arterial oxygen which would not usually constitute much stimulus to breathing suggests that either the abruptness of change in arterial oxygen is important or that some unidentified stimulus, itself sensitive to oxygen, is concerned.

Comparison of intraluminal pressures and motility patterns in the gastrointestinal tract of human beings when recorded by balloon and direct (electrical transducer) pressure systems. NICHOLAS C. HIGHTOWER, JR. (introduced by CHARLES F. CODE). *Mayo Fndn., Univ. of Minnesota, Rochester.*

Photokymographic records of gastrointestinal

intraluminal pressures and motility patterns have been obtained from fasted human beings by a direct pressure recording system employing a miniature electrical pressure transducer and by a water-filled balloon system. Simultaneous records have been made with both systems from various parts of the gastrointestinal tract with the miniature transducer and balloon in close proximity. Normal human beings were used as subjects for recording from the esophagus, stomach, upper part of the small bowel and the rectosigmoid. Patients with an ileac stoma were used for recording from the lower part of the small bowel and patients with a colonic stoma for recording from the colon. The position of the recording units was verified in each instance by means of fluoroscopy, roentgenography or both. A total of 56 records have been obtained from 33 subjects. In most parts of the gastrointestinal tract the simultaneous pressures recorded by the two systems were very nearly the same, agreeing, on the average, within ±4.0 cm. water. The motility wave patterns in the records obtained by the two methods were essentially the same in all parts of the gastrointestinal tract except those obtained from the gastric antrum where the balloon system sometimes detected waves not recorded by the transducer.

A comparison of spinal shock in normal dogs and in dogs made spastic by ischemia of the lumbar spinal cord. JOHN P. HILL* AND A. J. KOSMAN. *Depts. of Anatomy and Physiology, Northwestern Univ. Med. School, Chicago, Ill.*

The level of reflex activity in the lumbar spinal cord following low thoracic transection has been investigated electromyographically in a group of normal dogs and in a group of dogs whose hindlimbs had been rendered clinically spastic or rigid by ischemia of the lumbar spinal cord. It was found that in the normal animal the static stretch reflex was completely abolished for periods up to 72 hours and then returned only slowly; the crossed and flexor reflexes suffered a more prolonged depression. In the spastic animals, on the other hand, the static stretch reflex was never abolished completely and returned rapidly to pre-operative levels. While the crossed and flexor reflexes showed greater depression, they persisted in the majority of cases and were strongly present by 72 hours. The slight and extremely brief spinal shock has been closely correlated with an increasing loss of spinal inhibitory interneurons. These data have been interpreted as indicating that the development of spinal shock is dependent not only upon the loss of descending facilitory influences, but also upon the active participation of spinal inhibitory interneurons.

Statistical thermodynamics of free energy transfer in certain models of muscle action. TERRELL L. HILL* AND MANUEL F. MORALES. *Naval Med. Research Inst., Bethesda, Md.*

The essential requirements are discussed for a conversion of chemical free energy in ATP into mechanical work of contraction, in terms of theories of muscle action (for example, that of Morales-Botts) based on binding of ATP on myosin, shortening of myosin as a result of this binding, and removal of ATP from myosin by enzymatic dephosphorylation (in order to be able to repeat the binding process). According to this picture, the conversion of chemical free energy into work is accomplished by the over-all cycle but there is not a direct conversion in any one process in the cycle. A particular cycle illustrating these features is discussed thermodynamically with special reference to the free energy efficiency. Statistical mechanical models of an elastic fiber have been developed in order to apply the above thermodynamic analysis. These include a model of the Gergely-Laki type, one of the Astbury-Huggins-Pauling-Corey type and a network model (as in gels and rubber). In the latter 2 models electrostatic effects have been introduced explicitly by including the electrostatic free energy associated with charges on the fiber, in the presence of electrolyte. The statistical models predict the thermodynamic properties of the fiber, for example the change in length of the fiber at constant tension, as a result of binding ATP. The models also have applications to polyelectrolyte gels and fibers and to fibrous proteins in general.

Comparative effects of antiepileptic and other drugs upon forced circling produced by the intracarotid injection of di-isopropyl fluorophosphate (DFP). H. E. HIMWICH, I. H. WEINER,* ALAYNE COOMBS* AND EDITH CAMPBELL.* *Chemical Corps Med. Labs., Army Chemical Center, Md.*

Because forced circling produced by the intracarotid injection of DFP resembles a motor aura, it was used to test various drugs, Dilantin, Mesantoin, Phenobarbital, Meberal, Paradione, Trimethodione, and Phenurone, which were effective against convulsions. It was found however that they could not correct forced circling movements. In contrast, Caramiphen and Artane both prevented and cured forced circling.

An electrophysiological determination of tonotopic organization in auditory cortex of cat. J. E. HIND (introduced by W. D. NEFF). *Div. of Otolaryngology and Lab. of Physiological Psychology, Univ. of Chicago, Chicago, Ill.*

This study is based on the transient change in electrical potential which may be recorded at the surface of the exposed auditory cortex of a cat under barbiturate anesthesia in response to the onset of a pure tone. Comparison will be made of the results obtained by two methods: *a*) the nor-

mal cortical response, and *b*) the method of evoked strychnine potentials (TUNTURI. *Am. J. Physiol.* 162: 489, 1950). A description of the recording and stimulating instrumentation will be given, together with a brief discussion of the spectral nature of the acoustic stimulus required in this technique. Results to be presented include: *a*) the extent of the various cortical auditory areas (a search has been made for a third auditory area in the cat); *b*) examples of sharpness of tuning exhibited by small areas of cortex isolated by means of the evoked strychnine potential method; *c*) composite maps showing tonotopic organization throughout all auditory areas at threshold and suprathreshold intensities, together with an indication of the extent of individual variations.

Circulatory arrest in dogs following explosive decompression to 30 mm. Hg. F. A. HITCHCOCK, S. A. FRYE* AND J. P. KEMPH.* *Lab. of Aviation Physiology, Ohio State Univ., Columbus.*

When anesthetized dogs are explosively decompressed to 30 mm. Hg ambient pressure the mean arterial blood pressure drops within 15 seconds to a value of from 60–70 mm. Hg, and the pulse pressure decreases to zero. Usually there is no recovery from this condition until recompression. These results indicate that following explosive decompression complete circulatory arrest usually occurs. In an effort to determine the time at which circulatory arrest occurs 10 dogs were explosively decompressed to 30 mm. Hg and immediately after the decompression diodrast was injected into the external jugular vein and angiocardiograms were made at intervals of from 2–7 seconds for 25 seconds or longer. The circulation through the right heart, pulmonary vessels, left heart and aorta could be followed in this manner. Results showed that circulatory arrest occurred within 16 seconds in all dogs and within 10 seconds in 6 of the 10 animals. Eight of the dogs had gas in the heart within 10 seconds and in 1 other gas was found in 16 seconds. The Diodrast usually went no further than the right heart, although occasionally it was seen in the pulmonary veins or in the aorta and its branches. These data indicates that usually circulatory arrest which results from the formation of gas in the cardiovascular system occurs within 16 seconds after explosive decompression to 30 mm. Hg. Records of arterial blood pressure, however, indicate that occasionally such circulatory arrest does not occur until a minute or more after explosive decompression. In such cases the circulatory collapse is probably due to anoxia.

Effect of di-isopropyl fluorophosphate and of dibucaine on depressor responses evoked by topical application of methacholine chloride to the cerebral cortex. E. C. HOFF, E. D. SHACKELFORD,* E. H. SHARP,* L. E. RENNIE* AND E. L. KIRBY.* *Neurological Science Lab., Med. College of Virginia, Richmond.*

Transient falls of blood pressure produced by application of 2-mm. square pieces of filter paper soaked in methacholine chloride solutions to widespread loci on the convexity of the cat's cerebral hemisphere were obtained with more dilute solutions on the anterior sigmoid gyrus and gyrus proreus (0.62–1.25 mg/cc.) than on more posteriorly lying cortical areas (5.0–20 mg/cc.). The blood pressure depressions were of the order of 5–40 mm. Hg, occurring after a latency of 15–30 seconds and returning to the prestimulation base line in 20–30 seconds. Prior application (about 25 seconds beforehand) of di-isopropyl fluorophosphate (DFP) to a given cortical locus lowered the threshold for the depressor responses so that concentrations of methacholine chloride approximately one eighth those of control solutions were effective. Two to six minutes after local application of 2% dibucaine solution, the threshold for the depressor effect of methacholine chloride was similarly lowered. In experiments carried out on the sigmoid gyri and gyrus proreus (areas giving pressor responses to electrical stimulation) the pressor responses of a given locus to threshold electrical stimuli were unaffected by intercurrent depressor responses elicited from the same locus by methacholine chloride. Locally applied dibucaine abolished the electrical pressor responses but facilitated the methacholine chloride depressions.

Ultrastructure of the plasma membrane. JOSEPH F. HOFFMAN* AND JAMES HILLIER. *Princeton Univ. and the RCA Labs. Div., Princeton, N. J.*

The resolution afforded by the electron microscope has been used in an attempt to elucidate the molecular organization of the plasma membrane. Using successive centrifugations, human red cells were washed, hemolysed and washed further until as nearly free of hemoglobin as possible. The ghosts thus obtained were given a variety of treatments before and after mounting. The following tentative conclusions were drawn: *1*) The membrane is of the order of 50A thick and shows 2 levels of organization. *2*) It consists of a mosaic of fibers less than 40A wide, often appearing in small parallel groups with separations of the order of 100A. Double layers appearing as a cross-hatching are visible in some areas. *3*) There is a coarse mosaic of dense and light areas having dimensions in the range 500–1000A. The resulting 'texture' is fairly uniform in any cell but varies considerably from cell to cell in any one sample. *4*) Lipid extraction, osmium fixation, method of drying and many combinations of these produced no immediately obvious changes in structure at either level. *5*) Unfixed and osmium-fixed ghosts,

frozen-dried and examined stereoscopically, appeared completely empty.

Effects of DCA and cortisone on the adrenalectomized rhesus monkey. FREDERICK G. HOFMANN, ERNEST KNOBIL, AND THOMAS L. JONES (introduced by C. P. LYMAN). *Harvard School of Dental Medicine and Harvard Med. School, Boston, Mass.*

The effects of desoxycorticosterone acetate (DCA) and of cortisone on the symptoms of frank adrenal insufficiency in the bilaterally adrenalectomized male monkey have been studied. The blood levels of sodium, potassium, chlorides, inorganic phosphorus, nonprotein nitrogen, amino acid nitrogen and glucose as well as the hematocrit and body weight were determined at 3-day intervals. Appetite and general appearance were noted. Cortisone (2 mg/kg day for 6–12 days) failed to correct either the anorexia or the progressive loss in body weight observed following operation. Despite continued inanition, the blood glucose levels were dramatically returned to the control range. The blood concentrations of amino acid nitrogen and inorganic phosphorus returned to normal values, but the concentration of nonprotein nitrogen, although markedly reduced by cortisone, remained well above normal. Cortisone showed a distinct tendency to rectify the electrolyte imbalance in some animals. DCA (2 mg/kg day for 9–27 days) produced an improvement in appetite and gains in body weight were recorded although the blood glucose values remained low. The deranged blood sodium, potassium and chloride concentrations were quickly corrected; sodium values exceeding those of the preoperative period were sometimes noted. Inorganic phosphorus and amino nitrogen concentrations were consistently subnormal. Hemodilution was always observed. The efficacy of DCA and cortisone in relieving the symptoms of adrenal insufficiency is lessened as the severity of the deficiency symptoms grows more intense prior to institution of treatment. Irreversible structural damage may be implicated.

One-stage and two-stage methods for estimation of plasma anticephalin activity. R. R. HOLBURN,* R. T. CARROLL* AND L. M. TOCANTINS. *Dept. of Medicine, Jefferson Med. College and Hosp., Philadelphia, Pa.*

Variations in the response of different human plasmas to the clot accelerating action of human cephalin constitute the basis for measurement of plasma anticephalin activity. All work was done with silicone coated surfaces. In the rapid 1-stage method, anticephalin activity may be approximately estimated from the extent that incubation of the plasma with the cephalin reduces its thromboplastic activity, when compared with a similarly incubated normal plasma. In the 2-stage method, anticephalin activity can be measured in terms of the output of thrombin/minute from plasma activated by cephalin, as compared with normal plasma; the results are expressed in units. The mean anticephalin activity/ml. plasma in 28 normal plasmas was *4.81* U ± *2.28* (S. D.) (range *0.25–9.37*). In 10 hemophilics the mean was *16.4* U ± *3.8* (S. D.) (range *8.8–24.0*). Plasmas collected without special precautions or allowed contact with glass or asbestos become hypercoagulable and have a low anticephalin activity. Plasma to which a lipid antithromboplastin (*Proc. Soc. Exper. Biol. & Med.* 68: 110, 1948) has been added become hypocoagulable and have an anticephalin activity in the range found in hemophilic plasmas. When prothrombin or Ac-globulin are diminished, anticephalin activity is correspondingly exaggerated, since the amount of prothrombin and prothrombin conversion accelerators influence the rate of thrombin output in plasma activated by cephalin. Prolonged centrifugation increases anticephalin activity in the upper layers of the plasma and decreases it in the lower layers.

Fluid and changes in A–V shunt. J. H. HOLMES AND A. V. MONTGOMERY.* *Depts. of Medicine and Physiology, Univ. of Colorado School of Medicine, Denver.*

Plasma volume (T - 1824), available fluid (NaSCN), renal plasma flow (PAH), and glomerular filtration (creatinine) were determined on dogs before and after surgical production of an A-V shunt between the iliac artery and vein. These animals were followed for periods up to 2 years. Postoperatively there was either no change or a drop in weight. However, over a period of several months it gradually increased 1–2 kg. at which time the fluid available for dilution of thiocyanate had increased correspondingly (500–1500 cc.). The plasma volume increased 100–300 cc. at the same time or in 2 instances definitely 1 or more months after the increase in available fluid. The renal plasma flow and glomerular filtration rate showed either no change or a slight increase immediately after operation followed in several months by a progressive decrease in both values. Only minor changes were noted in the filtration fraction. Bone marrow studies showed inconstant evidence of increased erythropoiesis in the affected leg. Tissue electrolyte analysis of the affected leg when compared with the normal will be discussed. Placing of the shunt between the carotid artery and jugular vein offers an interesting comparison in physiological adjustment. Within a week there appears to be a rapid expansion in extracellular volume and a significant rise in renal plasma flow. The effects of stress as imposed by the rapid infusion of 1–1.5 l. of Locke's solution will be considered.

Salivary electrolyte excretion in man. J. H. HOLMES AND A. V. MONTGOMERY.* *Depts. of Medicine and Physiology, Univ. of Colorado School of Medicine, Research Lab., Denver Veterans Administration Hosp., Denver, Colo.*

During our investigation of thirst we became interested in the salivary secretion of electrolytes in man. The type of stimulus used for study can alter significantly the salivary volume and the electrolyte concentration independently. In 10 normal subjects, with mouth breathing the salivary volume averaged 1 cc./minute and the sodium concentration 7.7 mEq./l.; with citric acid stimulation the volume averaged 1 cc/minute, and the sodium concentration 17.1 mEq/l.; with paraffin gum the volume was 2.8 cc/minute and the sodium concentration 31.2 mEq/l. The potassium concentration remained relatively constant averaging 17 mEq/l. Following pilocarpine salivary flow increased 260%, and potassium and chloride concentrations decreased 25%. Epinephrine produced a 45% reduction in the volume but no significant change in the electrolyte concentrations. Pitressin produced a 60% decrease in volume, and a 30% elevation in the potassium concentration. In 26 patients treated with cortisone there were no statistically significant changes in the salivary electrolyte concentrations over a 3-week period (mouth breathing), though an occasional patient did show a drop in the sodium and chloride concentrations. Thiocyanate and radioactive iodine are excreted in the saliva at concentrations much higher than are found in the serum and when expressed on a clearance basis averaged 7.6 cc/minute, for thiocyanate and 7 cc/minute for I^{131} at low rates of flow. At higher rates of salivary flow (above .5 cc/minute) the I^{131} clearance ranged from 30–40 cc/minute and was not affected by the type of stimulus, the rate of flow, or changes in the chloride clearance.

Effect of propylene glycol on intravenous toxicity of dissolved compounds. RICHARD G. HORTON AND LILLIAN W. CONN.* *Chemical Corps Med. Labs., Army Chemical Center, Md.*

In determining the injection toxicity of many compounds, propylene glycol (PG) is a satisfactory vehicle due to its low toxicity, to its solvent action on many compounds soluble in either water alone or fat solvents alone and to the absence of hydrolysis of the solute. However, PG affects the toxicity of the solute when injected intravenously (i.v.) in small animals in the vol./kg. necessary for accurate measurement of doses. The necessary volume in mice is about 2.5 ml/kg. By itself, such a volume of PG injected via the lateral tail vein over a 4-second period produces severe transitory symptoms in most mice and very rapidly ensuing death in an average of about 6%. The i.v. toxicity to mice of various compounds in PG and in water has been compared to estimate whether PG affects their toxicity enough to preclude its use as the standard solvent of choice for i.v. toxicity work on small animals. Expressed as percentage, certain compounds were less toxic in water than in PG: DFP and NaCN, 20%; strychnine sulfate, 55%. Others were more toxic in water: atropine sulfate, 17%; nicotine alkaloid, 58%.

Effect of acute occlusion of the aorta upon cardiac output and hemodynamics. STEVEN M. HORVATH AND ROBERT W. HARRISON.* *Dept. of Physiology, State Univ. of Iowa, Iowa City.*

The ascending aorta of the dog has been occluded by means of a balloon forming an integral part of a catheter inserted through the femoral artery. Occlusions have been made at the fourth interspace for periods of time varying from 10 seconds to 20 minutes. Fifteen studies have been performed on 7 dogs. Pressure measurements were made simultaneously in the pulmonary artery, right ventricle, inferior vena cava, femoral artery and descending aorta. The cardiac output was determined by the Fick principle. These observations were made before, during and after the occlusion. The effects of varying degrees of interference with the blood supply to the lower two thirds of the animal will be considered. The alterations in the pressures observed in the animal during the early stages and immediately after the release of the occlusion will be discussed.

Movement of D_2O and Na^{22} across the frog renal tubule in the direction of capillary to tubular lumen. T. HOSHIKO,* R. E. SWANSON* AND M. B. VISSCHER. *Dept. of Physiology, Univ. of Minnesota, Minneapolis.*

The anatomical relations of the vascular supply in the amphibian kidney make possible a study of movement of materials across the renal tubule in the direction of capillary to tubular lumen. The glomerulus is supplied by the renal artery (RA) while the tubule probably by both the RA and the renal portal vein (RPV). The kidneys of 20 bullfrogs were doubly perfused—the RA with creatinine Ringer's and the RPV with inulin Ringer's containing D_2O (*ca.* 1 mole % excess) and Na^{22} (*ca.* 3000 cpm/cc.). Assuming that inulin enters the urine only by glomerular filtration, inulin clearance estimates RPV contamination of the glomerular filtrate. In most cases this was less than 1% of the total GFR. The urine specific activities for Na^{22} and D_2O were about 25 and 100 times greater than that predicted if the tubules were impermeable to the isotopes. Under this condition the urine specific activity would be equal to the glomerular filtrate specific activity. As a measure of the concentration ratio across the tubules, urine:renal vein (U/RV) specific activity

ratios were employed assuming that RV concentrations were peritubular capillary concentrations. These ratios were roughly 0.9 and 0.2 for D_2O and Na^{22}, respectively. The U/RV ratio for D_2O was consistently higher than that for Na^{22}, for any given clearance. Thus water and sodium moved from the capillary to tubular lumen at a time when net movement of both was in the opposite direction.

Experience with intermittent peritoneal dialysis for keeping alive dogs without kidneys. C. RILEY HOUCK. *Div. of Physiology, Univ. of Tennessee, Memphis.*

Bilaterally nephrectomized dogs were maintained by intermittent peritoneal dialysis and kept on the diet described by Grollman *et al.* (*Arch. Int. Med.* 87: 329, 1951). Plasma concentrations of the following were repeatedly studied: sodium, potassium, chloride, urea, NPN, creatinine, calcium, inorganic phosphate, phenols, and protein. In addition, plasma, total red cell, total blood and extracellular fluid (mannitol space) volumes; blood pressure; and body weight were frequently determined. Preliminary experiments warrant the following conclusions: *a*) Dialyzing in the morning and early afternoon followed by administration of homogenized diet by stomach tube 3 times during the late afternoon and evening was the best procedure. *b*) Vomiting and poor alimentation were related to use of the higher concentrations of glucose (3–10%) in the dialysis fluid and the timing and frequency of dialyses. Feeding and dialyzing could not be done simultaneously, because of vomiting associated with manipulation of the abdominal needle. *c*) Edema could be produced within a day or two by repeated dialyses with 0.75% glucose and substantially eliminated within 2 hours by one or more dialyses containing 5 or 10% glucose. *d*) Development of hypertension was independent of the level of the plasma constituents analyzed or changes in body weight. A high blood pressure once established was temporarily elevated following a blood transfusion. It rapidly returned to the pre-transfusion level and did not drop during suddenly imposed or gradual spontaneous reductions in body weight. (Supported by a grant from the American Heart Association.)

Certain physiologic factors involved in the production of right ventricular hypertrophy. HECTOR E. J. HOUSSAY* AND LEWIS DEXTER. *Med. Dept., Peter Bent Brigham Hosp. and Harvard Med. School, Boston, Mass.*

Factors influencing the production of right ventricular hypertrophy in man have been studied in *1*) patients with elevated right ventricular pressures with normal or reduced flows (inactive mitral stenosis, chronic cor qulmonale without cyanosis, and congenital pure pulmonic stenosis); *2*) normal right ventricular pressures with increased right ventricular blood flows (atrial septal defect). The criterion adopted for right ventricular hypertrophy was the presence of right ventricular hypertrophy or right bundle branch block by electrocardiogram. Right auricular mean pressure, as a measure of right ventricular dilatation, did not correlate well with these electrocardiographic changes. In mitral stenosis and chronic cor pulmonale, right ventricular hypertrophy appeared at a mean pulmonary arterial pressure of about 45 mm.Hg, but at a right ventricular mean systolic ejection pressure of only 30 mm.Hg in pulmonic stenosis, possibly because of the longer duration of the ventricular hypertension in the latter as compared with the former. Right ventricular hypertrophy occurred at a cardiac index of 7 l/min/m² in patients with atrial septal defect. Thus, right ventricular hypertrophy correlated well with either a high pressure or a high flow, while the over-all correlation with total pulmonary resistance and right ventricular pressure work was not uniform.

Analysis of the concentration curve in arterial blood resulting from continuous injection of dye. A. R. HOWARD,* PHILIP DOW AND W. F. HAMILTON. *Dept. of Physiology, Med. College of Georgia, Augusta.*

As reported to this Society last fall, dye injected at a constant rate into a stream perfusing the lungs yielded a curve reaching a plateau from which the flow could be calculated. When the injection was made into a normal dog's blood steam, recirculation usually commenced before such a plateau was reached. In the hands of ourselves and of others the curves occasionally showed apparent leveling off at a height that, on rare occasions, might yield figures to agree with the known blood flow. The leveling off might be due either to errors in analysis of successive samples or to such physiological accelerations of blood flow as would approximately cancel the natural buildup of the curve. To test these alternatives we used the logarithmic photoelectric densitometer and the polyethylene carotid loop cuvette developed in the laboratory of Dr. E. V. Newman. Intratracheal oxygen assured a steady oximetric base line in the nembutalized dogs. The curve was often completely devoid of plateaus. However if respiration was slow and deep a flattening or actual downward trend occurred with the respiratory acceleration of blood flow, as shown by corresponding rhythms at widely varying respiratory rates. These levelings off may occur at any height and bear no necessary relation to concentrations measuring blood flow.

Some effects of NaCl concentration on development of chick blastoderms in culture. EVELYN HOWARD. *Dept. of Physiology, Johns Hopkins Med. School, Baltimore, Md.*

The freezing point of egg white is accepted as equivalent to approximately 120 mM NaCl, in contrast to hen blood which is of the order of 160 mM. Disagreement as to the freezing point of egg yolk may have deflected attention from the biological significance of the activity of the water in egg white. The chick embryo proper, by the blastula stage, is separated from the yolk by a small pool of clear fluid in the blastocoele and lies applied to the non-cellular vitelline membrane, where diffusion from the white presumably impinges directly on the embryo. A study has been made of the effects of media at various concentrations in tissue cultures of chick blastoderms, explanted at the definitive streak stage. Mixtures of egg white and balanced saline, in agar, support partial differentiation of blastoderms for some thirty hours at both 120 mM and 160 mM, variations in total molarity being made with NaCl, other solutes being constant. At 120 mM, whole definitive streak blastoderms undergo a more nearly normal differentiation of general body form, develop more somites, more vigorous hearts with faster pulse rates nearer to the level *in ovo*, and more frequently develop true blood circulation than do embryos at 160 mM. Cultures of embryos explanted at slightly later stages show less difference between the two concentrations, as might be expected. The observations are consistent with the view that early embryonic tissues are adapted, presumably for phylogenetic reasons, to a medium more dilute than that of the adult.

Significance of the anion in active salt uptake by isolated frog skin. ERNST G. HUF, JOYCE WILLS* AND MARY JANE COOLEY.* *Med. College of Virginia, Richmond.*

Net active Na^+ uptake from .4 Ringer's by the epithelium of frog skin increased or decreased with increasing Na^+ concentration at the epithelial side, depending upon the kind of Na^+ salt added to .4 Ringer's. Controls were run without any supplements. NaCl, NaBr, $NaNO_3$, Na_2HPO_4, Na_2SO_4 increased, whereas Na I decreased, net Na^+ uptake. Analytical data showed that Br^-, I^-, NO_3^- and to a small degree phosphate and SO_4^- partly replaced Cl^- in the process of salt absorption. A sharp and considerable increase in p.d. of the skin followed the addition of Na I to the solution at the epithelial side ('outside solution'). This was not seen with other salts. The chorion was always positive relative to the epithelium. K^+ accumulation occurred in the outside solution and was greatest in the presence of Na I. A cation deficit was noted in the outside solution at the end of the experiments. The deficit was greater in the experiments than in the controls in the Na_2HPO_4 and Na_2SO_4 series. In the other series the opposite was found. The outside solution became more acid. Interpretations: Na^+ has the leading role in active salt uptake. Anions follow passively moving extracellularly and intracellularly. Br^-, ^-I and NO_3^- compete with Cl^- for transport and partly replace Cl^-. Phosphate and SO_4^- behave rather indifferently. I^- seems to encounter difficulties in moving intracellularly. As a result the skin potential increases. This makes it more difficult for Na^+ to move inwards, but it makes it easier for K^+ to move outwards. Some Na^+ moves inwards in a $Na^+ \rightleftarrows H^+$ exchange reaction. (A complete report will appear in *Pflueger's Archiv.*)

Drug effects on mammalian muscle spindles. CARLTON C. HUNT. *Wilmer Inst., Johns Hopkins Hosp., Baltimore, Md.*

Muscle spindles are specialized sense organs containing intrafusal muscle fibers which, in the mammal, are innervated by a separate group of efferent nerve fibers of small diameter. By their contraction they regulate the sensory responses of the spindle endings. The effects of drugs on this system were studied by recording the discharge in single afferent fibers from spindles to which one or more small nerve fibers had been isolated. Excitation of small efferent fibers caused no contraction in ordinary muscle fibers but increased the sensory discharge from the spindle depending on the amount of resting muscle tension. Intravenous injection of *d*-tubocurarine blocked the spindle response to small-nerve stimulation in doses which also blocked transmission in ordinary muscle fibers. This agent did not alter the spindle responses to muscle stretch; hence its effect is probably on transmission at the small-nerve-intrafusal muscle fiber junctions. Physostigmine facilitated the sensory response to small-nerve stimulation as well as producing the well-known increase in the tension response of ordinary muscle fibers to a single nerve stimulus. Intravenous injection of acetylcholine then caused a striking acceleration of the discharge frequency of spindle afferents although no contraction was produced in ordinary muscle fibers. This effect was blocked by the prior administration of *d*-tubocurarine. The findings indicate that junctional transmission in the small-nerve innervated muscle elements of the spindle is 'cholinergic.' The intrafusal muscle elements seem to have some pharmacological properties which differ from the 'ordinary' muscle fibers.

A quantitative demonstration of effect of variations in gastric emptying and secretion on acid-

ity of gastric contents. J. N. HUNT (introduced by M. O. LEE.). *Guy's Hospital, London.*

Differences in the acidity of the gastric contents after a meal are ascribed primarily to differences in the amount of acid secreted. The rate of gastric emptying is rarely emphasized as a factor influencing the acidity of the gastric contents. The effect of independent variations in emptying and secretion on the gastric acidity were studied *in vitro*. The Serial Test meal technique of Hunt and Spurrell (1951) *a*) showed the mean gastric secretion pattern of 20 students in response to a pectin test meal, and *b*) confirmed that a constant fraction of the gastric contents was evacuated/minute, i.e. emptying was exponential. The mean conditions in the stomachs of the 20 students were mimicked *in vitro* in a symphoning vessel. Acid was added at the proper rate from a burette. Under these circumstances emptying and the addition of acid were varied independently. It was found that doubling the fraction of the gastric contents evacuating/minute or doubling the mean rate of secretion ascertained for the group had almost equal effects on the gastric acidity.

Relationship between joint stiffness and synovial fluid characteristics. J. HUNTER, E. H. KERR* AND M. G. WHILLANS.* *Defense Research Med. Lab., RCAF Station, Toronto, Canada.*

Previous laboratory tests have shown that joint temperatures on exposure to cold fall to a greater extent than muscle, rectal or average skin temperatures. The fall in temperature is accompanied by an increased resistance of joints to movement. The present investigation of joint stiffness includes *1*) a study of the speed of flexion of the index finger before and after exposure to cold where the proximal interphalangeal joint was used. A decrement in speed was observed after exposure to cold; *2*) a consideration of the type of joint movement at the proximal interphalangeal and knee joints. x-ray studies of the knee joint establish the fact that the predominant movement of the tibia over the femoral condyles is a gliding one, and the same is true of the proximal interphalangeal joint; *3*) a study of bovine synovial fluid with particular reference to viscosity changes with temperature. Viscosity studies on synovial fluid show that the viscosity and the increase in viscosity for a definite fall in temperature are related to the mucin content. Departure of synovial fluid from Newtonian behavior is dependent on the mucin content and is quantitative. The characteristics of the fluid are such that a low rate of shear the viscosity is high and with a high rate of shear the viscosity tends to be less. The findings support the view that work effort is increased by the effect of low temperatures on synovial fluid.

Effect of nitrogen mustard on bakers' yeast. JOHN O. HUTCHENS AND BETTY PODOLSKY.* *Univ. of Chicago, Chicago, Ill.*

Bakers' yeast was exposed to 10^{-6}–10^{-4} M bis (β-chloroethyl) methylamine (HN-2). Optical densities of yeast suspensions were followed photoelectrically. O.D. vs. time curves were sigmoid and indicated that 3–4 cell divisions occurred before division was seriously inhibited (6–8 hours). Optical densities then increased very slowly for several hours after which the rate of increase returned to normal. Plate counts at various times indicated that the effects could be accounted for by the death of a large fraction of the cells after a few divisions. The cultures were then repopulated by the survivors. The curves superficially resemble the cell number vs. time curves reported by us in studies of effects of HN-2 on *Chilomonas*. However, with *Chilomonas* each cell is first inhibited and then recovers, few if any of the cells dying. Studies of respiration, carbohydrate, protein, and nucleic acid synthesis by yeast and chilomonads indicate that nucleic acid synthesis is first and most profoundly inhibited. Total nucleic acid synthesis was studied. Both cell types contain little DNA. The inhibitions noted must involve chiefly RNA though the possibility exists that DNA synthesis is more profoundly affected. In yeast, where the effects are lethal, decrease in nucleic acid content is detectable in 2–4 hours, i.e. before cell division slows markedly. At this time protein content is unaffected or slightly increased. With cessation of cell division at *ca.* 8 hours, the nucleic acid content/yeast cell is approximately halved while protein content is decreased *ca.* 20%. In chilomonads, which recover, the inhibitions are less profound and, since cell division virtually ceases, both nucleic acid and protein content/cell increase.

Effect of CO_2 on the analeptic action of amphetamine. D. E. HUTCHEON (introduced by L. B. JAQUES). *Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, Canada.*

In animals under barbiturate anaesthesia, CO_2 causes only a slight increase in respiration. To see whether amphetamine stimulates the respiration of dogs under deep Nembutal narcosis by restoring the sensitivity of the respiratory centre to CO_2, the following experiment was carried out. Dogs were deeply anaesthetized with Nembutal. The respiratory minute volumes were recorded by means of a spirometer. CO_2 in the expired air was altered either by changing the concentration of CO_2 in the inspired air in the spirometer by artificial respiration to blow off CO_2 which had accumulated as the result of the dressed breathing. The increase in ventilation produced by amphetamine sulphate (2.5 mg/kg.) was determined at various CO_2 concentrations (1–12%). It

was found that the increase in respiratory minute volume produced by amphetamine was less at high CO_2 concentrations than when the gas was reduced to normal limits. It therefore appears that the analeptic action of amphetamine is due to a direct stimulation of the depressed respiratory centre and that it does not act by increasing the susceptibility of the centre to accumulated CO_2.

Intrinsic factors affecting radiation mortality in the mouse. SIDNEY H. INGBAR AND NORBERT FREINKEL (introduced by CHARLES F. MORGAN). *Army Med. Service Grad. School, Walter Reed Army Med. Center, Washington, D. C.*

The influence of age, sex, weight and the duration of suckling on radiation lethality has been assessed in 600 mice receiving a standard dose of x-irradiation (550 air r., 9.0 ma., 120 KVP, 40 cm. target distance). Comparable control animals were observed. The age of the mouse at the time of irradiation influenced radiation lethality, regardless of body weight. No radiation deaths occurred in animals irradiated at 1 and 7 days of age. In animals irradiated at 15 days of age, a lethal effect became evident, increasing progressively in 22- and 30-day-old mice, and waning in 45-day-old animals. The mortality of postpubertal mice exceeded that of any prepubertal group. In postpubertal animals, the mortality in males was twice that in females. No such sex difference was evident in mice irradiated during the prepubertal period. Late deaths following weaning of mice irradiated at an early age were not seen. However, the mortality in 22-day-old mice weaned immediately following irradiation was greater than that of comparable mice allowed to suckle until 30 days of age. The effect of weaning at 22 or at 30 days of age upon the subsequent growth rates of both control and irradiated 22-day-old animals was determined. The time of weaning had no effect on the subsequent growth rates of nonirradiated animals. Growth rate ratios (irradiated/control) were reduced in the late postirradiation period in males weaned at 22 days, in contradistinction to ratios in males weaned at 30 days of age. Growth rate ratios in females of both groups exceeded those of the males. Similar sex differences in the effect of x-irradiation on growth rates were observed in mice irradiated at 1 day of age.

Experimental modifications of the differential permeability of the erythrocyte to ions. M. H. JACOBS, *Dept. of Physiology, Univ. of Pennsylvania, Philadelphia.*

The rate of exchange of radioactive for ordinary potassium and sodium by the mammalian erythrocyte is known to be measured in hours or days; that of small anions such as bicarbonate for chloride, in seconds. This highly characteristic differential permeability of the erythrocyte can be shown to be affected in a complex manner by *n*-butyl alcohol. With increasing concentrations and times of exposure, permeability to cations can be increased to almost any desired extent; similarly, permeability to anions may be progressively decreased up to a certain point, above which it is increased. Within a considerable range of conditions various combinations of opposing effects are possible, which may in part explain certain contradictory statements in the literature concerning the mechanism of hemolysis by alcohols and similar agents. In particular, it is possible to reconcile the view of Netsky and Jacobs (*Biol. Bull.* 77: 319, 1939) that butyl alcohol hemolysis, as ordinarily observed, is a consequence of swelling of a cell that is freely permeable to both anions and cations, with the finding of Ponder (*J. Gen. Physiol.* 32: 53, 1948) that in erythrocytes exposed to subhemolytic concentrations of butyl alcohol there may be considerable exchanges of sodium and potassium without significant volume changes of the cells. A further conclusion to be drawn from the observations here reported is that a simple system of electrically charged 'pores' like those postulated for certain artificial membranes is an inadequate explanation of the differential ionic permeability of the erythrocyte.

Specialized electromyography in supplement to clinical observations during hyperkinetic states in man ('functional nervous conditions'). EDMUND JACOBSON. *Lab. for Clinical Physiology, Chicago, Ill.*

In previous communications from this laboratory several forms of specialized apparatus of extreme voltage sensitivity and of relatively high stability have been described for recording neuromuscular states in man during health and under disordered conditions. A progress report can illustrate how carefully controlled methods can be employed to supplement clinical observations during hyperkinetic disorders, the so-called 'functional nervous disorders' in man. Photographic or pen recordings of action-potentials in electrodes in selected skeletal muscular sites can provide briefer records when required, but for recordings during periods such as 30 minutes or longer, the integrating neurovoltmeter provides values of potential differences which can be plotted in mv. against time. Characteristically patients when agitated show relatively high values from the 4 regions which I have generally recorded, namely in one biceps-brachial group, one quadriceps femoris muscle, one eye-brow region and one side of the jaw. Patients of quieter disposition or those under effective doses of certain sedatives show lower values as a rule. Among the lowest values typically found are those in persons trained to

relax. It is concluded that with further improvement of methods of recording physiological states the investigation and the handling of 'nervous disorders' can assume a more scientific status.

Steroidal studies on patients with adrenal virilism. JOSEPH W. JAILER AND JEAN LOUCHART.* *College of Physicians and Surgeons, Columbia Univ. and Presbyterian Hosp., New York City.*

This report deals with some aspects of a study of adrenal virilism. The administration of ACTH results in an increase in the urinary 17-ketosteroid values in a majority of cases of virilism as it does in normal individuals. Fourteen patients with the adrenogenital syndrome due to bilateral adrenal hyperplasia were then given either cortisone or Compound F for various periods of time. In all 14 patients there was a dramatic fall in the total neutral 17-ketosteroid excretion values within 4 to 5 days, from the elevated levels characteristic of this disease to amounts approximately normal for the age and sex of the patients. These results confirm the reports of Lewis and Wilkins; Bartter, Albright *et al;* and Jailer. It would appear that secretory activity in adrenal hyperplasia is dependent upon pituitary stimulation and that cortisone and Compound F suppress ACTH production, resulting in inhibition of adrenocortical secretion. However, in 2 patients with adrenal tumors the administration of cortisone was without effect upon the level of urinary 17-ketosteroids. Adrenal neoplasms appear to be independent of pituitary stimulation and consequently even in the presence of pituitary inhibition there is no decline in the urinary 17-ketosteroids. The urinary neutral 17-ketosteroids have been fractionated by column chromatography according to the method of Devis and steroidal patterns in adrenal hyperplasia before and after cortisone and also in adrenal masculinizing tumors will be presented.

Inhibition of acid secretion in dogs by the carbonic anhydrase inhibitor, #6063. HENRY D. JANOWITZ, HENRY COLCHER* AND FRANKLIN HOLLANDER. *Gastroenterology Research Lab., Mount Sinai Hosp., New York City.*

The recent synthesis of a new group of heterocyclic sulfonamide compounds with marked carbonic anhydrase inhibitory power suggested a study of their effects on the gastric secretion of acid. One of these substances, 2-acetylamino-1,3,4-thiadiazole-5-sulfonamide (compound #6063) was selected because it had been shown to be 440 times as potent as sulfanilamide for the 100% inhibition of carbonic anhydrase *in vitro*, and effective in inhibiting renal acid formation *in vivo* (Berliner *et al.*). Three dogs with vagally denervated fundic pouches were employed. Acid secretion was stimulated by s.c. injection of 0.025 mg. histamine base every 10 minutes. After an appropriate control period, a single injection of compound #6063 was given intravenously. In the dose range 20–60 mg/kg. body weight, there was marked inhibition of acid secretion in 40–60 minutes in all animals. Volume of juice, concentration of acid, and thus output of acid fell markedly. Inhibition of HCl output varied between 70 and 97% of control values, regardless of dosage within this range. It persisted for at least 3 to 6 hours. The secretory response to histamine had recovered after 24 hours in several experiments. These injections of the compound were well tolerated. That this inhibition of acid secretion depends solely on the inhibition of carbonic anhydrase in the parietal cell is purely inferential at present. (Acknowledgment is made to Dr. R. O. Roblin Jr., American Cyanamid Company, for generous supplies of compound #6063.)

Critical flicker fusion in the rat. HARRY J. JERISON,* M. G. GOLDZBAND* AND GEORGE CLARK. *Dept. of Psychology, Univ. of Chicago, Dept. of Anatomy, Chicago Med. School, Chicago, Ill.*

Although the measure of critical fusion frequency has been found clinically useful, the lack of knowledge of the basic physiology for the phenomenon has limited its application. We have devised a method of this determination of critical fusion frequency in the rat. The driving mechanism for flicker is an electronic multivibrator which supplies current to a neon tube. The on and off periods are equal regardless of the frequency. A behavioral test is used in which the rat differentiates between a flickering and a steady light. Fusion thresholds of pigmented rats and human subjects were obtained. Flicker rate versus intensity functions in these species is similar though, at the intensities used, human fusion occurs at a flicker rate about 20 cps. above that for rats. In a series of 10 rats frontal brain damage did not appreciably alter the CFF.

Blood sugar levels and hypoxia. HOWARD A. JEWELL (introduced by HARRY A. CHARIPPER). *New York Univ. Grad. School of Arts and Science, New York City.*

After approximately 80% of the pancreatic material of male rats has been removed, the animals do not exhibit diabetic blood sugar levels. The animals gain weight normally and their blood sugar levels (nonstarved or after 24 hours' starvation) fall within normal range. Normal animals and these partially pancreatectomized animals were placed in a low pressure chamber for various periods under a pressure comparable to 25,000 ft. The change in blood sugar levels after this stress were dependent upon whether or not the animal was starved. An increase in blood sugar level was

found in all the normal nonstarved rats after 1 and 3 hours. After 6 hours, however, the blood sugar fell back to its original level, whereas blood sugar levels of the nonstarved partially pancreatectomized animals were high and frequently approximately (25%) within diabetic ranges. Some of the levels in these animals increased more than 100 mg. %. These high levels were temporary and disappeared within 24 hours. Even after repeated treatment, the partially pancreatectomized rats did not exhibit a permanent hyperglycemia. Rats which had been starved for 24 hours showed an initial high sugar level after being subjected to lowered atmospheric pressure, but after 6 hours exhibited a lowered level. The blood sugar levels of partially pancreatectomized starved rats remained depressed after 1, 3 and 6 hours. Preliminary investigations indicate that alloxan diabetic animals do not manifest comparable blood sugar changes.

Some vascular responses in the dog to *l*-epinephrine and *l*-nor-epinephrine. KENNETH E. JOCHIM. *Dept. of Physiology, Univ. of Kansas, Lawrence.*

Simultaneous registration of pressure and flow pulses in the unopened carotid artery of the dog by means of the electrical capacitance manometer and electromagnetic flowmeter, respectively has permitted the study of the pressure/flow ratio as an index of vascular resistance in the carotid bed. *L*-epinephrine and *l*-nor-epinephrine in dosages ranging from 0.5 γ/kg. to 25.0 γ/kg. uniformly produced a decrease in mean carotid blood flow and an increase in carotid vascular resistance as indicated by a rise in the mean pressure/mean flow ratio. Depressor effects caused by small doses of *l*-epinephrine were also accompanied by a significant reduction in flow and an increase in resistance. Flow effects of both drugs consistently lasted longer than effects on blood pressure. The magnitude of the constrictor effect of each drug varied roughly with the initial value of the blood flow; no conclusions were possible regarding the relative constrictor activity of the 2 drugs. Dibenamine in amounts sufficient to reverse the pressor effect of *l*-epinephrine had little effect on its constrictor action. (Supported by a U. S. Public Health Service grant.)

Kinetics of D_2O exchange in perfused organs. JOHN A. JOHNSON AND H. MEAD CAVERT (introduced by H. B. STEINBACH). *Dept. of Physiology, Univ. of Minnesota, Minneapolis.*

The isolated dog heart and dog gastrocnemius muscle have been perfused with blood to which D_2O has been added. The concentration of the D_2O in the arterial blood was kept constant and measurements were made of blood flow as well as of D_2O concentration in venous blood and tissue water. It was found over the range of flows studied (25–49 cc/min/100 gm. of heart, 3–22 cc/min/100 gm. of skeletal muscle) that, to a fair approximation, blood flow rather than permeability factors governs the over-all rate at which blood D_2O exchanges with the extravascular water. Thus, the concentration of D_2O in the outflowing blood, C, followed the relationship $C=C_0\ (I-E^{-kt})$ in which C_0 is the concentration in the inflowing blood and K is the ratio of the flow of blood water to volume of tissue water. The results also indicate that the time for exchange of D_2O to equilibrium between the red blood cells and the plasma is less than the period during which the blood remains in an average capillary (of the order of 1 second).

Contractile system of smooth muscles. WILLIAM H. JOHNSON* AND JOHN F. PERKINS, JR. *Dept. of Physiology, Univ. of Chicago, Chicago, Ill.*

A method has been described previously (PERKINS AND JOHNSON. *Federation Proc.* 1950, 1951) for continuously measuring the mechanical resistance ('viscosity') and the elasticity of smooth muscles during contraction. A sinusoidal displacement is applied to the muscle through a light spring and the phase angle between the motion of the spring and of the muscle is measured together with the amplitude of these motions. This information allows us to calculate the above coefficients. The method has now been extended so that a greater range of frequencies can be used, and an electronic phase meter has been constructed to facilitate the analysis of the data. Using this improved method, we are undertaking to measure the variation of the mechanical resistance and elasticity as the frequency of displacement of the muscle is changed. Tobolsky and Eyring (*J. Chem. Physics* 11, 1943) have shown that in high polymer systems in general, a theoretical relation exists between the frequency dependence of the mechanical resistance and the number of secondary bonds within the material. We hope to use this relationship to detect any change which might occur as the muscle contracts in the secondary bonding within the contractile system. By this means it may be possible to tell whether the muscle is contracting by an increase in total intermolecular bonding or whether the bonds are being broken within the contractile system so as to allow the contractile protein to shorten entropically.

Trypsin inhibitor in pancreatic juice. MARTIN KALSER (introduced by A. C. IVY). *Dept. of Clin. Science, Univ. of Illinois College of Medicine, Chicago.*

Studies were carried out to determine whether the trypsin inhibitor known to be present in pancreatic tissue is secreted into the pancreatic juice. A method for assaying trypsin inhibitor in canine

pancreatic juice has been devised, consisting of *1*) adding an equal volume of 5% trichloracetic acid to the pancreatic juice, *2*) heating in a water bath at 80° C. for 5 minutes, *3*) filtering, *4*) extracting the filtrate twice with ether, *5*) placing under vacuum until the residual ether is driven off, and *6*) bringing the filtrate to pH 7 with NaOH. The inhibitor titer was determined by adding an equal volume of the filtrate to a solution of crystalline trypsin, allowing to stand at 5° C. for 30 minutes, and then determining the trypsin activity by the hemoglobin substrate method of Anson. A control was run using water treated in the same manner as pancreatic juice. The difference in trypsin activity between the control and test solutions gives a quantitative estimation of the amount of inhibitor present in the pancreatic juice. All samples of pancreatic juice examined so far have been found to contain inhibitor. The inhibitor prepared in the above manner resembles the crystalline trypsin inhibitor of Kunitz and Northrop in that it was not precipitated by 2.5% trichloracetic acid, was not found in tissues other than the pancreas, and was dialyzable through a cellophane membrane. When pancreatic juice was first incubated with a duodenal powder extract and the activated juice was then extracted for trypsin inhibitor, it was found that the amount of inhibitor was diminished.

Thirst and excretion after salt administration. GERLAD S. KANTER (introduced by E. F. ADOLPH). *Dept. of Physiology, Univ. of Rochester, Rochester, N. Y.*

Responses to loads of NaCl administered by stomach tube were studied in 6 female dogs. The concentrations of the solutions varied from .15M–.50M, and the dosage was 2% of body weight. Water was freely available for drinking. Quantitative collection of urine was through an implanted metal bladder cannula. As the input concentration increased, the animals increased their drinking in a linear fashion. When body fluid shifts were calculated, the intracellular space appeared to be maintained within 3% of control while the extracellular space varied over a range of +7%–+15%. Diuresis was observed at higher input concentrations. The average chloride concentration in the urine increased as the input concentration increased, but it did not approach the maximal urinary concentration possible. Two general regulatory patterns in diverse dogs under the same stress were observed. Minimal internal regulation is manifested by dogs, which with a positive salt load and the opportunity to ingest tap water, drink to the point at which they excrete urine nearly isotonic in respect to chloride. Maximal internal regulation is shown by dogs which drink less water and use the kidneys to correct chloride imbalance of the body by secreting hypertonic urine. This distinction is a convenient means of viewing the 2 regulatory processes involved in recovery toward isotonicity.

Regulation of breathing during exercise. FRED KAO* AND FRED S. GRODINS. *Dept. of Physiology, Northwestern Univ. Med. School, Chicago, Ill.*

Theoretical analysis has shown that the forcing of the ventilatory regulator during exercise must consist of both a parametric component (increased gas exchange) transmitted humorally, and a direct component ('exercise stimulus') whose pathway of transmission is unknown. Cross-circulation experiments were performed to test the alternative hypotheses of neural versus humoral conduction of the direct component. The hind limb of one dog ('neural dog') was perfused exclusively by blood from a second dog ('humoral dog'). Exercise (steady state) was induced in the cross-perfused limb of the neural dog by direct electrical stimulation of the muscles. In a total of 12 experiments on 6 such preparations, it was found that in the humoral dog, both ventilation and oxygen consumption increased but the ventilatory equivalent for oxygen decreased (parametric forcing). The neural dog, however, showed an increase in ventilation with no change in oxygen consumption so that the ventilatory equivalent increased (direct forcing). The behavior of the neural dog thus differed from that of the humoral dog, and both differed from the intact animal in which ventilation and oxygen consumption increased proportionally so that the ventilatory equivalent remained constant (parametric + direct forcing). The latter was true whether the muscles were stimulated directly or via spinal motor roots (13 experiments). These results are incompatible with the hypothesis of exclusively humoral transmission of the direct component, and qualitatively compatible with the hypothesis of its exclusively neural conduction. (This investigation was supported in part by a research grant from the National Institutes of Health, Public Health Service.)

Epicardial unipolar electrograms from the dog's right atrium. ANTHONY A. KATTINE (introduced by JOHN C. SCOTT). *Dept. of Physiology, Hahnemann Med. College, Philadelphia, Pa.*

Unipolar electrograms were taken from unstimulated hearts and also from those responding to reflex vagal stimulation. The recording electrode consisted of cotton thread soaked in normal saline and was either stitched to the epicardium or set in place. The electrical circuit was set so that when the recording electrode was relatively positive an upward deflection was produced; and when relatively negative a downward one was produced. Components of the atrial complex are described

by terminology used by Levine *et al.* (*Am. Heart J.* 37: 46, 1949). A QS_a wave was obtained when the recording electrode was on the S.A. node region and also at points on the Superior Vena Cava. On atrial muscle an R_a wave was recorded which increased in height as the electrode was moved away from the S.A. region. The R_a wave was followed by an S_a wave which at all points on atrial muscle was more prominent than the R_a except at points remote from the S.A. region. At these points the R_a often equaled the S_a. In no tracing was a Q_a wave obtained. The S_a-T_a segment was elevated in the S.A. region and fell to the base line in records taken from areas below the S.A. region. Simultaneous records from 2 points showed that under reflex vagal stimulation the area from which QS_a waves were obtained expanded and often included areas below the S.A. region. With stimulation R_a waves were often recorded from the upper limits of the S.A. region where previous to stimulation QS_a waves were obtained.

Effect of dietary deficiencies on prostatic response of the albino rat to testosterone propionate. JOHN W. KEARNS,* JOHN T. GRAYHACK* AND W. W. SCOTT. *James Buchanan Brady Urological Inst., Johns Hopkins Hosp., Baltimore, Md.*

A study was made of the effect of general dietary deficiencies on the response of the castrated male rat to testosterone propionate. Previous observations on castrated Wistar strain rats subjected to reduced intake of stock diet, or a diet consisting only of vitamin-free casein or only of U.S.P. glucose and given varying doses of testosterone propionate had revealed a decreased prostatic response in the animals on restricted dietary intakes at the higher dosage levels of testosterone propionate (2 mg/day). Despite the marked weight loss in the experimental group, the prostatic growth of the deficient and control animals was approximately equal at the lower dosages of testosterone propionate (0.05 mg/day). A second series of experiments was then undertaken in which a modification of the Cannon diet (4C) was used as the control diet and groups of castrated animals were given this diet from which one major food component, such as protein, fat, carbohydrate, vitamins, salt or liver extract, had been omitted. Four weeks after castration and 3 weeks after these animals had been on the special diets, injections of 4.0 mg. of testosterone propionate every other day were begun. Two days after the eighth injection the animals were sacrificed. The animals on deficient diets all failed to attain the weight of controls with the exception of those on a diet deficient in fat. Protein deficient diet caused the greatest weight loss. Animals fed diets deficient in glucose and protein showed the least prostatic response to testosterone propionate.

Significance of the carbon dioxide tension in urine. THOMAS J. KENNEDY, JR.,* JACK ORLOFF* AND ROBERT W. BERLINER. *Section on Kidney and Electrolyte Metabolism, Natl. Heart Inst., Natl. Insts. of Health, Bethesda, Md.*

Data from 195 urine samples collected in 43 experiments from 14 normal dogs have been analyzed to establish the range of variability in the CO_2 tension in urine and to formulate a hypothesis for the interpretation of this variability. A direct relationship between the pCO_2 and the concentration of buffer other than bicarbonate was observed. The CO_2 tension was most sensitive to buffer concentration when the urine pH was high, least sensitive when the urine pH was low. At low values for urine pH, CO_2 tensions lower in urine than in arterial plasma were regularly noted. An inverse relationship existed between the pCO_2 and the urine flow. These relationships obtained in urine whether collected from renal pelvis or from bladder, and were observed after presumed abolition of hydrogen ion secretion by administration of inhibitors of carbonic anhydrase. The data will be discussed in terms of the hypotheses which have been offered to explain previously published and similar observations. It will be shown that the data conform closely to predictions from the hypothesis that diffusion equilibrium for CO_2 is reached within each nephron, and that subsequent mixture of moieties of disparate pH and buffer concentration in a region in which surface-volume relationships are less favorable to the back diffusion of CO_2 produce the observed CO_2 tension of bladder urine.

Absence of immediate effects of x-radiation on gastric secretion. HERBERT D. KERMAN,* T. P. DE GRAFFENRIED II,* F. J. BAJANDAS,* F. E. COY, JR.* AND W. S. REHM. *Depts. of Radiology and Physiology, Univ. of Louisville School of Medicine, Louisville, Ky.*

Numerous investigations have been reported in which gastric acidity has been suppressed by the actions of x-rays, gamma rays of radium, and the beta radiation of P_{32}. These studies indicate that there is a definite latent period following irradiation before a significant decrease in gastric acidity becomes apparent. However, because of the differences in techniques of irradiation and the methods used to determine secretory activity of the gastric mucosa, there is a paucity of experimental evidence of the immediate effects of ionizing irradiation on gastric mucosa. Utilizing the chamber technique of Rehm (*Am. J. Physiol.* 141: 537, 1944) to measure the rate of secretion of hydrochloric acid and the potential difference across the stomach wall, a portion of the stomach in 6 dogs was

irradiated and the secretory rate and potential difference were measured at regular intervals for 3 hours. Low voltage irradiation was delivered to the portion of the stomach in the chamber, with a beam of a HVL of 0.9 mm. of aluminum and a HVL of 0.3 mm. of aluminum. Dosages were in R/air 1000 (2 dogs), 55,000 (1 dog), and 155,000 (3 dogs). There were no significant effects on the secretory rate or on the potential difference. Results of these experiments would seem to corroborate further the observations of many investigators that a latent period exists following irradiation before demonstrable functional changes occur.

Effect of ovariectomy and of certain sex hormones on islet weight. ESME H. KERR* AND R. E. HAIST. *Dept. of Physiology, Univ. of Toronto, Toronto, Canada.*

The effects of gonadectomy and of injections of oestradiol benzoate, stilboestrol and progesterone on islet weights in intact rats were studied. Islet volumes or weights were estimated by the method of Haist and Pugh. The islet weights in rats ovariectomized for 25, 53, and 140 days were not significantly different from those of pair-fed control animals. Oestradiol benzoate (Frosst) injected into female rats in the amounts of 4 mg. in 3 weeks, 8 mg. in 6 weeks and 12 mg. in 6 weeks occasioned an increase in the mean islet weights as compared to those of pair-fed controls, though this difference was shown to be not significant statistically. Injections of 30 mg. of stilboestrol a week for 6 weeks into rats caused a significant increase in the weights of the islets of Langerhans as well as in the islet weights/100 gm. body weight. Injections of 30 mg. progesterone/week for 6 weeks caused an increase in the mean islet weight as compared to the pair-fed controls, but this was shown to be not significant statistically.

Influence of pH upon blood flow and peripheral resistance in hind leg of dog. NANCY C. KESTER,* ALFRED W. RICHARDSON* AND HAROLD D. GREEN. *Dept. of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.*

In general little attention has been paid to the pH of solutions tested for vasomotor effects. Having observed that intra-arterial injections of sodium pentobarbital, which has a very high pH, resulted in marked vasodilatation, we decided to investigate whether or not the vasodilator effect could be due to the pH of the solution. We prepared a four-component buffer solution with a pH range of 2–11. One ml. quantities of this solution, adjusted to various pH values, were injected intra-arterially in the hind leg of a dog while recording flow with an electromagnetic flowmeter. A minimum change in flow was observed with solutions of pH 7.4 (115% of control), but as the pH was changed in either direction flow increased, so that at pH 2.0 it was 217% of control and at pH 10.0 it was 234% of control. The estimated change in blood pH produced by the injection at pH 2.0 was −0.87 and by the injection at pH 10.0 was +0.70. Larger changes in blood pH were required on the acid than on the alkaline side to produce comparable increases in flow. The results suggest that a physiologic mechanism exists which is designed to restore homeostasis of $[H+]$ or $[OH^-]$ concentration with disturbance on either side of physiologic range. (Supported by grant H-487(C2) from the National Heart Institute.)

Veratrine electrical responses of muscle treated with anticholinesterases and with sodium lack. GERALDINE KIEBEL (introduced by HARRY A. CHARIPPER). *Washington Square College of Arts and Science, New York University, New York City.*

In mechanical studies of responses of veratrinized (10^{-6}) frog sartorii (*Anat. Rec.* 108: 125, 1950), it was found that the duration and magnitude of the veratrine contracture were considerably reduced by the addition of eserine (0.00154 M). The electrical changes associated with this effect have now been studied, using d.c. recording. Responses have been registered at 5-minute intervals, single shock stimulation starting 5 minutes after the local application of veratrine or eserine-veratrine solution to the region of only one of the pickup electrodes. Each eserine-veratrine response exhibits an initial action potential tetanus, followed by a contracture potential of considerably shorter duration and smaller amplitude than that of the pure veratrine responses. The electrical changes thus parallel the mechanical. When prostigmine, a nonpenetrating anticholinesterase, is used in place of eserine, no significant change in the typical response occurs. Thus, the results suggest that the presumed inhibition of the cholinesterase system by eserine may accelerate membrane repolarization during veratrine contracture. In muscles equilibrated to a Ringer's having 80% of the sodium replaced by choline, local application of veratrine resulted in responses showing *1*) always a single spike only, and *2*) variable effects on the contracture depolarization, with regard to which more work is needed for clarification. Thus, a clearly indicated effect of Na-lack is at least to suppress the tetanus behavior characteristic of the veratrine response. (Aided by Contract NR113-300 of the Office of Naval Research.)

Sensitive method for assaying vasoactive substances in the rat hindlimb. W. R. KINGSOLVER,* A. DONTAS* AND S. W. HOOBLER. *Dept. of Medicine, Univ. of Michigan Med. School, Ann Arbor.*

A simple, sensitive and inexpensive technique

for measuring minute amounts of vasoactive substances in the intact animal is described. It has the advantage of utilizing the animal's own blood at a constant perfusion pressure. A miniature T-cannula is inserted into the abdominal aorta with its distal limb reaching into one femoral artery and side arm leading through polyethylene tubing to a pressure bottle. For each determination 0.2 ml. of blood is allowed to enter the side arm, the proximal aorta occluded, and 0.1 ml. of saline control or vasoactive substance introduced into the proximal segment of the side arm. Constant pressure is then applied to the fluid in the side arm, forcing blood into the femoral bed. Rate of flow is measured by the time required for a blood-bubble interface to traverse a segment of the tubing. Normal flow to the hind-limb is reestablished by releasing the aortic clamp. Vascular resistances may be calculated by dividing perfusion pressure by flow rate. The procedure may be repeated once a minute for 1–3 hours. Arterial or vascular end pressures may also be obtained with the same preparation. The minimal doses to produce resistance change were compared with the intravenous doses required to alter the blood pressure of the anesthetized rat. With nor-epinephrine significantly increased resistances occurred at .0025–.005 μg., while significant pressor effects were obtained with .025 μg., intravenously. The minimal intra-arterial dose of methacholine to decrease resistances was .0005–.001 μg. (Supported by a grant from the Michigan Heart Association.)

Tracer studies on carbonate pool of dairy cows. MAX KLEIBER. *College of Agriculture, Univ. of California, Davis.*

Intravenous injection of C^{14} labeled carbonate into lactating cows and analysis of the milk constituents showed that 10% of the carbon in the lactose, 4% of the carbon in the casein and 2% of the carbon in the fat originated from carbonate in the bloodstream (KLEIBER, SMITH, BLACK. *J. Biol. Chem.* In press). Infusion of C^{14} labeled carbonate into a cystern of a cow's udder indicated that a considerable part of the incorporation of carbonate-carbon into lactose occurs in the udder itself. The corresponding results for casein suggest that all the labeling of amino acids proceeded via blood carbonate. These latter results were, however, inconclusive because the radio activity was low, and similar trials with higher doses are in preparation. About three fourths of the injected C^{14} is given off as respiratory CO_2 in 140 minutes after intravenous injection and in 170 minutes after udder infusion of labeled carbonate. An analog computer has been constructed to deal with tracer problems that involve more than 2 pools and thus lead to differential equations, whose integration is difficult. We hope that this machine will enable us to express our results of carbonate metabolism in cows in terms of pool capacities and turn over rates.

Modifiability of the diurnal body temperature and heart rate curves. NATHANIEL KLEITMAN. *Dept. of Physiology, Univ. of Chicago, Chicago, Ill.*

During the continuous daylight of the arctic summer, at Tromsö, Norway, a family of 3 women and 1 man followed artificial sleep-wakefulness schedules, with appropriate adjustments of activity and meal hours. First, an 18-hour routine was adhered to for 3 weeks, then there were 2 weeks of 'normal' living, followed by 3 weeks on a 28-hour routine. Each artificial schedule necessitated sleeping around the clock, but the depressing effect of having, at times, to carry on 'daytime' activities in the dark was absent. The new routines disrupted the regular diurnal body temperature curve of the women, but, although there were individual differences in adaptation, definite 18- or 28-hour curves were not developed. The man who had been the subject of similar tests in the past showed, as previously, a complete fixity of the 24-hour body temperature curve on both the 18- and 28-hour schedules. By contrast, the heart rate, observed on 2 subjects, including the temperature-fast one, promptly conformed to the 18- and 28-hour routines, acquiring distinct new curves. It thus appears that the diurnal body temperature curve is a rhythm not easily modified by a change in routine, whereas the usually quite parallel heart rate curve would seem to be a simple periodicity, immediately determined by the particular schedules of hours of sleep, activity, and meals. (Aided by a grant from the Dr. Wallace C. and Clara A. Abbott Memorial Fund of the University of Chicago.)

Spinal pain conduction by chains of short neurons. M. KLETZKIN* AND E. A. SPIEGEL. *Dept. of Experimental Neurology, Temple Univ. School of Medicine, Philadelphia, Pa.*

The return of pain sensation following bilateral interruption of the long pain conducting spinal pathways points to accessory pain transmitting systems. Extraspinal conduction of visceral pain is suggested by the persistence of pain from the human urinary bladder after transverse lesions of the cord, and has been demonstrated for the gall bladder of cats (Ashkenaz in this laboratory). These experiences necessitated a re-evaluation of Schiff's experiments in which pain conduction from the sciatic to supraspinal centers persisted after crossed hemisection of the cord. Such experiments give positive results (increase in depth and frequency of respiration) only if a large number of segments lie between the 2 hemisections. Under these conditions (with one hemisection in the lower thoracic and the second in the upper cervical

cord on the opposite side) one of us (Spiegel) elicited pain reactions in cats by injecting barium chloride into the femoral arteries. Extraspinal conduction of painful stimuli from the somatic system (sciatic stimulation) or from the pelvic viscera (stimulation of the pelvic nerve) is in itself insufficient to produce the respiratory effects since transverse section of the thoracic cord abolishes this reaction, while lumbar sympathectomy in the cross-hemisected animal does not. The changes in respiration appearing on stimulation of somatic as well as of sacral vegetative nerves in the cross-hemisected animal seem to demonstrate spinal pain conduction by chains of short neurons. (Aided by a grant from the National Institute of Mental Health of the National Institutes of Health, Public Health Service.)

Measurements of life-span of white blood cells with radioactive phosphorus. DANIEL L. KLINE AND EUGENE E. CLIFFTON (introduced by GEORGE R. COWGILL). *Dept. of Physiological Chemistry and Section of Oncology, Dept. of Surgery, Yale Univ. School of Medicine, New Haven, Conn.*

Two and one-half mc. of radioactive phosphorus was administered by mouth as inorganic phosphorus to a selected group of patients who had normal and total differential white blood counts and who were free of diseases known to affect the white blood cells. Blood samples (50 ml.) were collected at intervals after administration of the isotope. The red blood cells were separated from the leucocytes by the addition of fibrinogen. Desoxyribose nucleic acid (DNA) was isolated from the white blood cells, and the specific activity of the DNA phosphorus (counts/min/mg. P) was determined. The curve of isotope concentration against time shows a slow increase during the first 4 days following administration of P^{32}, then a rapid addition of labeled cells to the circulation. A plateau level was maintained from the 8th to the 12th day which was followed by a decline in the specific activity of the DNA phosphorus of the white cells along an S-shaped curve to the 22nd day. This series of events is qualitatively almost identical with that obtained by other workers who estimated the life-span of red blood cells by measurement of the incorporation of glycine labeled with N^{15} into hemin. Mathematical analysis of the data indicates an average life-span of about 13.5 days for the white cells. The cells appear to remain out of the circulation during the first 4.5 days after their formation. These data represent an average of the various white cell types. It is probable that the curve obtained is a composite of at least 2 components, the lymphocytes and neutrophils.

Role of adrenal glands in the plasma and urinary electrolyte changes during moderate and severe anoxia. R. F. KLINE. *Dept. of Physiology, Univ. of Maryland School of Medicine, Baltimore, Md.*

Exposure of cats to moderate and severe anoxia indicates not only involvement of the adrenal gland in electrolyte changes noted, but also shows an antagonistic action between the medullary and cortical divisions of the gland. The adrenal cortex exercises dominant control at moderate altitudes such as 28,000 ft. This control is shown by the 18, 19, and 14% decrease in plasma potassium noted in normal, nephrectomized and splanchnicectomized animals, respectively, following a 90-minute exposure to 28,000 ft. When, however, animals were adrenalectomized or nephrectomized-adrenalectomized and exposed to like conditions, no significant reduction in plasma potassium resulted. These decreases as well as observed increases in total urine potassium (51%) and volume (88%), give a true picture of the action of cortin. Exposure of normal and nephrectomized animals to altitudes above 40,000 ft., resulted in increases of 58 and 69%, respectively. When, however, adrenalectomized and splanchnicectomized were exposed to like conditions, the plasma potassium increased only 18 and 11%, respectively. Thus, the influence of the adrenal medulla predominates at higher altitudes, causing large as well as rapid increases in plasma potassium values which are similar to those caused by the injection of adrenaline.

Effect of diet upon food and water intake during a survival period under temperate conditions. R. F. KLINE AND H. C. DYME.* *Aero Med. Lab., Wright Air Development Center, Wright-Patterson Air Force Base, Dayton, Ohio.*

In basic studies of physiological relationships for use in formulating survival ration requirements, experiments were conducted to determine the effect of various dietary regimens on water consumption and fuel intake of rats over a 14-day period. Water consumption on carbohydrate diets was found to be fairly low and in some cases less than in starving controls. On a fat regimen the water intake was also low. The water intake on a protein diet was high compared to the water consumption on a balanced ration. The fuel intake of rats with free access to water when on a diet consisting of carbohydrates was excessively high as compared to the intake of animals on fat or on protein. Thus it appears that carbohydrates and fats are preferable components for single-constituent diets in situations where water supply is restricted; while the feeding of protein under such conditions is contraindicated. Furthermore, animals subsisting on single-constituent fat or protein

regimens restrict their food intake while animals on carbohydrate regimens markedly increase their food intake.

Adrenal insufficiency in the rhesus monkey. ERNEST KNOBIL,* FREDERICK G. HOFMANN,* THOMAS L. JONES* AND ROY O. GREEP. *Harvard School of Dental Medicine and Harvard Med. School, Boston, Mass.*

A comprehensive study has been made of the effect of single-stage bilateral adrenalectomy of the rhesus monkey on changes in concentration of various blood constituents, body weight, appetite, general bodily appearance and behavior, and on the length of survival following operation. Preoperative control data were obtained for each monkey. The animals exhibited severe anorexia, progressive loss of weight, weakness and final collapse (10–16 days following the operation). These symptoms were accompanied by a constant increase in serum potassium and an unsteady decline in both serum sodium and chlorides. Blood glucose reached a minimum level (30–40 mg. %) near the middle of the survival period and remained there until death. Nonprotein nitrogen rose steadily in the manner characteristic of other species; the peak value (210 mg. %) was noted in the animal with the longest survival period. A consistent rise in blood inorganic phosphorus was observed. Blood amino acid nitrogen concentration increased following adrenalectomy, achieved a maximum around the middle of the postoperative period, and tended to return to the control level preterminally. Hematocrit values fell sharply immediately following the operation, and gradually returned to normal before death. Hemoconcentration was not evident in any instance and the animals did not die in a shock-like state. Autopsies failed to reveal gastrointestinal lesions or any other gross pathological changes. These data were accumulated to serve as points of departure in evaluating the efficacy of cortical steroids in a species whose affinity to man lends special meaning to the results.

Influence of hypothalamic and thalamic lesions upon the action of nociceptive impulses and CO_2 on the electroencephalogram. WERNER P. KOELLA (introduced by E. GELLHORN). *Lab. of Neurophysiology, Dept. of Physiology, Univ. of Minnesota, Minneapolis.*

Previous experiments by Gellhorn and collaborators (*Federation Proc.* 146: 630, and *Arch. f. d. ges. Physiol.* 1952) have shown that nociceptive stimuli and relatively low concentrations of carbon dioxide induce excitation in hypothalamus and cortex as shown by action potential studies. This effect seemed to indicate a primary action of these factors on the hypothalamus and a secondary excitation of the cortex via hypothalamic-cortical pathways. The following experiments have been performed in order to test this interpretation. In 19 anesthetized (Dial or Pentothal) and curarized cats the effect of nociceptive stimuli and CO_2 (10% and 15%) on the ECG before and after uni- and bilateral coagulations in different areas of the hypothalamus and thalamus has been recorded. Unilateral lesions in the posterior hypothalamus usually diminished or even blocked ipsilaterally the excitatory action of the above mentioned stimuli. After bilateral coagulation in the posterior hypothalamus the response of the cerebral cortex to these afferent stimuli and CO_2 was diminished or eliminated. Lesions in the anterior hypothalamus, however, failed to produce such an inhibitory effect upon the action of nociception and CO_2. In some instances the excitatory action of these stimuli was even greater than before coagulation. After destruction of the medial thalamus the changes were similar to those seen after posterior hypothalamic lesions. These experiments seem to show the importance of the integrity of the hypothalamic-thalamic-cortical pathways for bilateral cortical excitation in response to nociception and to inhalation of CO_2. (Aided by the Office of Naval Research.)

Chemical changes in the blood in children with lipoid nephrosis before, during and after treatment with ACTH (Corticotropin). BENJAMIN KRAMER, DANIEL D. CASDEN,* HERBERT GOLDMAN* AND SAMUEL SILVERMAN.* *Pediatric Research Labs., Jewish Hosp. of Brooklyn, N. Y.*

Studies on the blood of 12 children suffering with lipoid nephrosis were carried out before, during and after treatment with Corticotropin. The following determinations were made: total protein; A/G ratio, both by chemical determination and electrophoresis; total lipids; cholesterol and cholesterol partition; erythrocyte sedimentation rate; sodium; potassium; calcium; inorganic phosphorus; bicarbonate; chloride; urea. The following changes were observed either preceding or following diuresis (the values quoted refer to average pre- and post-treatment levels): a decrease in total lipid from 2212 mg.%–840 mg.%; a decrease in total cholesterol from 776 mg.%–533 mg.%; in most instances the free and combined cholesterol decreased proportionately; a rise of the total protein from 4.9 gm.%–5.6 gm.% with an increase in the albumin fraction from 1.7 gm.%–3.1 gm.% and a fall in the globulin fraction from 3.2 gm.%–2.5 gm.%; hypokalemia was observed in 2 instances; otherwise there were no striking changes observed in the sodium, potassium, chloride or bicarbonate; the elevated erythrocyte sedimenta-

tion rate tended to fall and in 6 cases reached normal values. In the absence of diuresis, the changes were neither as consistent nor as marked. With one exception the abnormal blood chemistry did not return to normal. The significance of these findings is discussed.

Calorigenic activities of thyroxine and thyroglobulin. ROBERT L. KROC AND NEIL R. STASILLI (introduced by A. A. HELLBAUM). *Research Dept., Chilcott Lab., Morris Plains, N. J.*

Dose-response curves for subcutaneously administered *dl*-thyroxine and a soluble thyroglobulin preparation were determined in normal and thyroidectomized male rats. Injections were made on 3 consecutive days. Basal oxygen consumption was measured daily before, during, and following the treatment period. Food was restricted to 5 hours each day. Based on chemical determination of 'thyroxine,' the thyroglobulin extract was more active metabolically than crystalline thyroxine: 2.5–3 times in normals and 3–8 times in thyroidectomized rats depending upon the levels of BMR increase compared. Thyroidectomized rats were responsive to both preparations over a wide range of low dosages which had no measureable calorigenic action in normals. In this dosage range the curve for thyroidectomized rats was asymptotic at response levels of 15–30%. The oxygen consumption in this range of metabolic increase was characteristic of normal controls of the same age. At higher dosage levels the difference in responsiveness of thyroidectomized and normals gradually disappeared. With thyroglobulin the curves merged at BMR increases approximating 50%. With thyroxine this was observed at about 30%. The curves for thyroglobulin plateaued at about 65% increase in BMR in contrast with about 50% for rats receiving thyroxine. The steepest portions of the curves obtained with normals had slope values about twice those obtained with thyroidectomized rats. The interpretation of data based on chemical analysis for 'thyroxine' in extracts and the use of normal rats for bioassay of thyroglobulin and thyroxine will be discussed.

Carbon dioxide pressures in the alveoli during the respiratory cycle. HUGO KRUEGER. *Oregon State College, Corvallis.*

It has been tacitly assumed that alveolar carbon dioxide pressures remain constant under resting or other steady state conditions. Yet simple calculations indicate that there is a significant variation in the carbon dioxide pressure in the alveoli during each respiratory cycle. Let us assume a residual air of 2000, an expiratory reserve of 1500, a tidal air of 500 and a dead space of 150 ml. with a partial pressure of 40 mm. Hg for carbon dioxide in the alveolar air at the end of expiration and a carbon dioxide production of 4 ml/second. At the end of expiration the dead space is filled with alveolar air. The volume of carbon dioxide in the alveoli at the end of expiration would be given by $3500 \times 40/713$ or 196 ml. The volume of carbon dioxide in the dead space would be given by $150 \times 40/713$ or 8 ml. At the end of inspiration the volume of air in the alveoli would be $2000 + 1500 + 500$ or 4000 ml. At a respiratory rate of 12/minute, inspiration would last about 2.5 seconds and 10 ml. of carbon dioxide would be delivered to the alveoli by the blood. The alveolar carbon dioxide would be $196 + 8 + 10$ or 214 ml. The pressure would be $214 \times 713/4000$ or 36 mm. Hg. Thus alveolar carbon dioxide pressure varies from 36–40 mm. Hg during each respiratory cycle. This corresponds to a change of 0.05 pH in the blood. (Basic data from DOUGLAS AND HALDANE, *J. Physiol.* 45: 235, 1912.)

Urinary sodium excretion in normal guinea pigs. HUGO KRUEGER, ROSALIND WULZEN, DARRELL DAVIS* AND LLOYD YONCE.* *Oregon State College, Corvallis.*

Guinea pigs, previously on a diet of greens, barley and salt cake, when placed in a metabolism cage for 36 hours with water *ad libitum* but no food, initially produce small quantities of urine and infrequently at a rate near 0.5 cc/hour. In some guinea pigs this situation maintains for 36 hours; but usually, 12 or more hours later, there is a marked increase in the rate of urinary water elimination (2, 4, 6 and 9 cc/hour maxima), and about 4 hours subsequently a decrease toward the initial rate (previously interpreted as a night-day-night low-high-low pattern. *Am. J. Physiol.* 163). The rate of sodium excretion is also low initially (below 0.3 mg/hour) and increases abruptly (maxima 1.1, 9, 6, 9 mg/hour) before or concurrently with the water rate increase. The sodium excretion rate does not fall as rapidly as the water excretion rate. With no food or water sodium intake, an increased absorption of sodium from the gastrointestinal tract is unlikely. The explanation is suggested that the high metabolic rate of the guinea pig leads to a high net destruction of tissue cells after available food reserves are used up. This would release the accompanying extra-cellular water and Na. When subjected to the severe metabolic stress of a 36-hour hunger period, the guinea pig initially conserves water and sodium; later water and sodium are excreted more rapidly but it is not clear why the rate of sodium excretion increases more rapidly than that of the water.

Survival of sympathetic nerve fibers during chronic electrical stimulation. W. G. KUBICEK, G. GULLICKSON,* M. E. OLSON* AND F. J. KOTTKE. *Div. of Physical Medicine, Univ. of Minnesota Med. School, Minneapolis.*

Unilateral silver electrodes imbedded in lucite

blocks for support were placed around the lumbar sympathetic nerves of growing puppies. Approximately 2 weeks later stimulation was started with sinusoidal alternating current (3 cps) 20 hours/day. This routine was continued for about 6 weeks. Histological studies were made on the nerve tissue under the electrodes and compared with similar unaltered fibers on the opposite side. Apparently normal numbers of fibers were present under the electrodes. Occasionally ganglion cells were found under the electrodes. These cells appeared normal. There was survival in both myelinated and unmyelinated fibers. The results of the histological studies correlated well with physiological response. Acute stimulation produced a decrease in skin temperature of the leg on the same side. Chronic stimulation decreased the rate of bone growth in the rear leg on the stimulated side. Lack of physiological response was usually accompanied by severe histological damage of the nerve trunk or by complete sloughing due to displaced electrodes. Summary: With careful placement of shielded silver electrodes sympathetic nerve fibers and ganglion cells can survive at least 6 weeks of chronic electrical stimulation. The assistance of Dr. Berry Campbell in the histological interpretations is gratefully acknowledged.

Incomplete neuromuscular transmission in twitch system of frog's skeletal muscles. STEPHEN W. KUFFLER. *Johns Hopkins Univ., Baltimore, Md., and Marine Biological Lab., Woods Hole, Mass.*

Maximal stimulation of the nerve to the frog's submentalis muscle, *in situ* or in isolation, causes a twitch in which not all muscle fibers contract. Such submaximal contraction is due to incomplete transmission at the junctions. Transmission can be enhanced in the following manner: *1)* by repetitive nerve stimulation and *2)* by increasing the stretch on the muscle. A similar effect of stretch on transmission has recently been reported (LIBET *et al. Biol. Bull.* 101: 199, 1951). In preparations which are confined to a strip of the submentalis muscle, normal endplate potentials (e.p.p.'s) can be observed without first fatiguing, curarizing, or otherwise treating the junctions. Stretch enhances transmission by increasing the e.p.p. size without appreciably altering its time course. E.p.p.'s which were previously subthreshold for muscle excitation thereby become adequate for excitation. Repetitive stimulation acts by summing e.p.p.'s until they reach the size which is required for transmission. The effect of stretch in increasing e.p.p.'s has also been studied in fatigued and curarized sartorius muscles, providing a basis for the anti-curare action of stretch. The frog possesses a 'small-nerve' transmission system in which nerve impulses caused graded contractions at the junctions (KUFFLER, S. W. AND R. W. GERARD. *J. Neurophysiol.* 10: 383, 1947), and a twitch system where 'large-nerve' fibers set up propagated impulses. The latter system was hitherto believed to operate with a large safety margin, the nerve impulse-muscle impulse ratio always being 1:1. The present studies show that 'transmitter' production at various junctions in many frog muscles may vary over a wide range.

Effect of hyperoxygenation on rate of blood flow in perfusion of bovine mammary glands. SOMA KUMAR,* S. LAKSHMANAN,* E. A. CORBIN* AND J. C. SHAW. *Dairy Dept., Univ. of Maryland, College Park.*

One of the chief difficulties encountered in the perfusion of bovine mammary glands has been that of obtaining a normal rate of blood flow. In an attempt to solve this problem a systematic study was made to determine the factors involved. In a typical perfusion the initial rate of blood flow is fairly high but decreases with the progress of the perfusion. It was noted that the blood became highly oxygenated towards the latter part of the perfusion due to its repeated passage through the oxygenator. Preliminary observations indicated that the rate of blood flow was associated with the degree of oxygenation. In 4 different perfusion experiments the mammary glands were perfused alternately with poorly oxygenated and highly oxygenated blood. In each case perfusion with highly oxygenated blood resulted in a marked decrease in the rate of blood flow within a minute or two. Replacing the highly oxygenated blood with the poorly oxygenated blood restored the rate of blood flow with the same rapidity. For example, in one experiment an initial rate of 600 ml. of blood/minute was obtained with poorly oxygenated blood. With the replacement of the poorly oxygenated blood with highly oxygenated blood the rate of blood flow dropped to 200 ml/minute within a minute. The original rate of blood flow was obtained immediately when the poorly oxygenated blood was used. These results were obtained when pure oxygen was used for the oxygenation of blood. When oxygen containing 5% of carbon dioxide was used the effect was not observed. Further studies demonstrated that the addition of chloral hydrate to the blood overcame the apparent vasoconstriction caused by the blood hyperoxygenated with pure oxygen. It appears that the vasoconstriction and dilation effects under the conditions noted above are nervous in origin.

Effect of variations of oxygen and carbon dioxide tensions in inspired air on latency of blackout produced by pressure on the eyeball. E. H. LAMBERT AND H. BJURSTEDT.* *Mayo Fndn., Rochester, Minnesota, and Karolinska Inst., Stockholm, Sweden.*

Intra-ocular pressure was increased by pressing on the eyeball through the lower lid with a Bailliart tonometer. Pressure above that required to collapse the retinal arteries and cause eventual complete loss of vision was used. The mean latent period between application of the pressure and the onset of dimming of vision for 10 subjects breathing air was 4.7 ± 0.15 seconds. The latent period was increased on the average to 5.4 seconds breathing 60% oxygen and 6.4 seconds breathing 100% oxygen. These changes were highly significant, statistically, and suggest that under these conditions the latent period is related to the quantity of oxygen dissolved in the tissues rather than to oxygen saturation of hemoglobin. In one experiment breathing oxygen at 2 atmospheres pressure further prolonged the latent period. Breathing 10% O_2 reduced the latent period only temporarily to 4.3 seconds ($P < 0.05$) but within 3 minutes the latent period was on the average 4.7 seconds even though oxygen saturation of hemoglobin continued to fall. Five % carbon dioxide in air increased the latent period to 5.2 seconds, while a similar concentration in oxygen increased the latent period to 7.8 seconds. These changes are in harmony with the effects of anoxia and hypercapnia on retinal blood flow. Similarity of blackout produced by eyeball pressure and positive acceleration suggests that breathing oxygen and carbon dioxide may increase g tolerance by increasing the latency of symptoms beyond the time required for pressor reflexes to produce effective compensation.

Retention of vitamin B_{12} after administration to subjects of different ages. CALVIN A. LANG AND BACON F. CHOW (introduced by ANNA M. BAETJER). *Dept. of Biochemistry, School of Hygiene and Public Health, Johns Hopkins Univ., Baltimore, Md.*

A small portion of the vitamin B_{12} administered parenterally to normal adult rats in dosages of 1–2 μg. appears in the urine, and the remainder is retained by certain tissues and concentrated in organs such as liver, kidneys, and pancreas. Experiments were carried out to compare the urinary excretion and retention of this vitamin after injection into rats of different ages. For this purpose, 15 2-month-old and 16 14-month-old male rats raised on our stock diet fortified with 2% desiccated whole liver were placed in individual metabolism cages. They were divided into 4 subgroups and given a single subcutaneous injection of crystalline vitamin B_{12} tagged with Co^{60} in dosages of 1–8 μg/rat. Urine specimens (0–24 and 24–48 hours after administration and in some instances pre-injection samples) were collected and assayed for vitamin B_{12} both by a microbiological method and by radioactivity measurement. Fourteen days after injection the animals were sacrificed, and the above-mentioned organs were removed for the determination of radioactivity after conversion into inorganic Co^{60} salts. Our results demonstrate: *1)* At a given dosage the excretion of vitamin B_{12} was essentially the same for both age groups if expressed as μg/animal but was significantly different if expressed as μg/100 gm. body weight. This is due to the fact that the average weight of the older animals (457 gm.) was approximately twice that of the young ones (248 gm.). *2)* Retention was related both to dosage and to age. Similar excretion studies were made using human male subjects of 2 different age groups, namely, 25–40 years of age and 65 or greater, who were of essentially the same body weight. Our preliminary results again indicated that the young individuals excreted more.

Mechanism of adrenal cortex reaction to stress. L. L. LANGLEY, P. W. SCOKEL* AND J. A. WHITESIDE.* *Dept. of Physiology, Med. College of Alabama and Univ. of Alabama School of Dentistry, Birmingham.*

It is well established that a wide variety of stress conditions causes increased activity of the adrenal cortex. Two possibilities have been suggested. There is evidence to indicate that the first response of the organism is a nervous activation of the adrenal medulla. Epinephrine is then thought to stimulate the anterior lobe of the pituitary to liberate ACTH which, in turn, activates the adrenal cortex. A slower, but perhaps more important mechanism pictures the stress conditions causing an increased utilization of adrenal cortical hormone; as a result the blood level of this hormone decreases, causing an outpouring of ACTH which activates the adrenal cortex. Using rats exposed to low atmospheric pressures we find both liver glycogen accumulation and adrenal ascorbic acid values to be good indicators of adrenal cortical activity. With this technique it has been demonstrated that the adrenal medulla does play a role in the stress reaction. It has also been found that so long as hypocapnia is prevented there is no adrenal cortical activation. This suggested that the disturbed acid-base balance may be an essential link in the sequence of events set off by stress. This possibility is supported by the observation that nephrectomized rats give no indication of adrenal cortical activation in response to stress. It is suggested that in the normal animal the disturbed acid-base balance is compensated for by renal function which utilizes abnormal quantities of adrenal cortical hormone. As a result ACTH is liberated and the adrenal cortex activated.

Unconditioned orogastric secretory reflex. K. J. LANGLOIS,* R. K. S. LIM, GENE ROSIERE,*

D. I. STEWART* AND D. L. STUMPFF.* *Physiology Dept., Creighton Univ., Omaha, Nebr.*

Blondlot's explanation of the gastric secretory response to eating as a gustatory (orogastric) reflex, although accepted by Pavlov as the unconditioned component of the cephalic phase of gastric secretion, has never been verified experimentally. Pavlov's sham feeding procedure in adult animals elicits both the unconditioned and conditioned components. To avoid the latter, the gustatory nerves were stimulated in dogs under chloralose. Faradic or thyratron stimulation (minimal for cardiac inhibition) was applied during alternate minutes for 15–120 minutes (usually 30–45 minutes). A small gastric secretory response was obtained on stimulation of the IXth (9 of 14 experiments) and VIIthCT (2 of 7 experiments) nerves. The latent period became shorter (15 minutes or less) with repeated stimulation. The response usually outlasted the stimulus by 15–45 minutes, with the peak sometimes occurring during the after discharge. The high incidence of negative results and the low response suggested the possibility of inhibitory influences. In 3 dogs with the diencephalon and internal capsules sectioned through or above the hypothalamus, stimulation of the IXth and XthP (pharyngeal) nerves, and insulin (1.3 U/kg.) gave minimal gastric responses; stimulation of the VIIthCT gave no response. After midbrain or pontine section (leaving about 2 mm. uncut ventrally) in 5 dogs, stimulation of the VthB (buccinator), VIIthCT, IXth and XthP nerves, and insulin (1.7 U/kg.) resulted in large responses, (comparable with sham feeding after VIIthCT, IXth and insulin stimulation). The orogastric reflex center in the medulla, besides being excited by afferent (gustatory) impulses, is susceptible to chemical stimulation by insulin hypoglycemia, and is subject to inhibition from tegmental and/or hypothalamic areas.

Motoneurone excitability in man following afferent nerve volleys: intact subjects and patients with upper motor neurone lesions. HELEN W. LANGUTH,* ROBERT D. TEASDALL* AND J. W. MAGLADERY. *Subdept. of Neurological Medicine, Johns Hopkins and Baltimore City Hospitals, Baltimore, Md.*

Motoneurone excitability in man can be directly tested in certain spinal segments by monosynaptic H reflexes (*Bull. Johns Hopkins Hosp.* 88: 499, 1951). The single nerve shocks now employed excite low-threshold afferent fibers but not motor axones. In intact subjects a similar preceding shock to the same nerve is immediately followed by facilitation decaying over 15 to 25 msec. Succeeding suppression lasts for 100 msec. or more. Excitability then rises, before secondary depression lasting several hundred msec. ensues. Stronger volleys produce no initial facilitation; suppression is immediate and prolonged. In patients with upper motor neurone lesions (cerebral or spinal) well above the segment tested, conditioning volleys produce similar facilitation then suppression if weak; solely suppression if stronger. In these patients, however, motoneurone excitability begins to recover earlier, i.e., in 35 to 60 msec. after weak volleys and only somewhat longer after stronger ones. Moreover recovery is more complete, or excitability may even be enhanced, before secondary depression (comparable to that in intact subjects) appears. The findings suggest that the functional changes created by these clinical lesions may occur not directly at motoneurones but at internuncial levels. They are consonant with the previous postulate that much, at least, of the prolonged suppression found in normal man operates through internuncial pathways.

Electric countershock as a cardiac defibrillator. HARLAN E. LAPE,* FRANK G. MILLS* AND GEORGE L. MAISON. *Dept. of Pharmacology, Boston Univ. School of Medicine, Boston, Mass.*

Cardiac resuscitation from fibrillation in the dog was successfully accomplished by means of shocks of alternating current, direct current and condenser discharge. Bare stainless steel electrode discs measuring 4.6 cm. in diameter were used. Ventricular fibrillation was produced by applying low voltage a.c. current to the heart of the etherized dog. A massage rate of 25 compressions/minute proved most efficient for the dog heart as measured by production of maximal arterial blood pressure. Massage and epinephrine alone (3 μg/kg/min. continually infused up to 35 minutes) did not defibrillate the heart in any of 12 cases. With a.c. countershock serial defibrillation (10 rapid volleys within 10 seconds) at 240 volts (approximately 3 amp.) was found to be the most effective voltage for defibrillation, namely 9 of 10 cases. Of 76 fibrillating hearts which were eventually returned to rhythmic beating by countershock, only 2 passed through a demonstrated phase of cardiac arrest. This low incidence was attributed to careful maintenance of normal femoral MAP by massage and the resulting adequate oxygenation of the myocardium. In hearts successfully defibrillated, direct current countershock produced more severe burns on the myocardium than either a.c. or condenser discharge countershocks. A single condenser discharge countershock (128 μfd. charged at 1000 to 1500 volts, discharged through the heart alone) resuscitated the fibrillating heart in 77% of 31 cases. Of 46 cases resuscitated by a.c. countershock, 20 survived (24 hours or more); of 5 cases resuscitated by d.c. countershock, 1 sur-

vived; of 28 cases resuscitated by condenser discharge, 12 survived.

Simultaneous comparison of two injection methods for cardiac output. HAMPDEN C. LAWSON, W. F. CANTRELL,* J. E. SHAW* AND D. L. BLACKBURN.* *Depts. of Physiology and Pharmacology, Univ. of Louisville School of Medicine, Louisville, Ky.*

The dye T-1824 was added to suspensions of autogenous erythrocytes containing cellular radiophosphorus, and the mixture was injected through catheters into either the right or the left ventricle of barbitalized dogs. The total volume of mixture injected was usually about 4 ccm., containing approximately 2 mg. of dye, and 20μc. of P_{32}. Samples were collected from a femoral artery for plasma dye concentration, as previously described, at the rate of about 2 samples/second, for about 30 seconds following the injection. From the other femoral artery blood was drawn at a constant rate through thin-walled tubing coiled about a Geiger-Müller tube connected with a scaling unit. A continuous record of pulse-frequency in the scaler was obtained from an Esterline-Angus recorder, by an arrangement of condensers and a relay. A description of the recording apparatus and its calibration for the present purposes, is given elsewhere. Each injection of the mixture permitted two independent calculations of cardiac output. In general, there was excellent agreement, usually within 5%, between the value obtained by calculation from the dye data and that obtained by calculation from the P_{32} data, with random direction of disagreement.

Free longitudinal vibrations in aortic strips. RICHARD W. LAWTON AND ALLEN L. KING.* *Dept. of Physiology, Cornell Univ. Med. College, New York City and Dept. of Physics, Dartmouth College, Hanover, N. H.*

Frequencies of free longitudinal vibrations of aortic strips were measured as a function of applied load and relative length L/L_o, where L_o is the initial length. The curves of $\omega^2 L_o/g$ versus L/L_o are comparable to those obtained from rubber. They have distinct minima that become sharper and move upward and toward the $\omega^2 L_o/g$ axis as the specimens become stiffer. The experimental curves are described by theoretical equations derived from current relations for the extension of an ideal elastomer; they may be used for determining values of the elastomeric parameter β (*J. Applied Physiol.* 22: 1340, 1951). Values of the parameter are somewhat higher under dynamic conditions as compared to static conditions. Deviations of experiment from theory due to creep, beating and hysteresis in the tissue strips are noted. Tentative calculations indicate that normal function of the aorta takes place in the neighborhood of the minimum.

Nutritional factors in hemodynamics: hypertension during PGA administration in albino rats. RICHARD E. LEE, ELIZABETH A. HOLZE* AND SACHIKO TANAKA.* *Dept. of Medicine, New York Hosp., New York City, and Natl. Heart Inst., Bethesda, Md.*

Albino rats on a synthetic complete ration were given intraperitoneal injections of pteroylglutamic acid (PGA) 1.0 mg/100 gm. of body weight three times weekly, for 6 weeks. After 3 weeks, and again after a total of 6 weeks of treatment, their mean mercury carotid blood pressure was compared to that of litter mate controls of the same sex receiving the same synthetic ration. Liver, kidney and adrenal ascorbic acid was measured, and sections were prepared from the kidneys of certain animals. Animals given PGA for 3 weeks had 'normal' blood pressures. Following a 6-week period of injections, mean blood pressure in the PGA-treated animals was significantly elevated above that of controls, and punctate fibroblastic scars were present in the renal cortex. There were no renal tubular casts, nor evidence of tubular obstruction, and kidney weights were comparable to those of untreated animals. A slight but insignificant elevation of liver ascorbic acid was found in the treated rats. Possible hypertensive mechanisms of PGA are discussed.

Mechanism of glucose transfer across the human red cell surface. PAUL G. LEFEVRE AND MARIAN E. LEFEVRE.* *College of Medicine, Univ. of Vermont, Burlington.*

Earlier observations concerned with the kinetics of the penetration of the human erythrocyte by glucose were extended to include glucose exit from the cell as well as its entry, with the widest variety in the relations between several quantitative factors contributing to the glucose gradient and resultant osmotic volume changes. For example, the initial cell volume or glucose level, the extracellular glucose concentration, or the initial gradient, or the total glucose transfer or volume change, or other such factors, were each held constant while other factors were varied, and the volume changes recorded (Ørskov method). Provided the glucose level in the medium did not exceed about three-fourths isosmotic, all records showed patterns compatible with a 'carrier' system involving reversible complex-formation between glucose and a cell surface component, by simple mass action. The complex must be highly undissociated and the reactions much faster at the outer than at the inner interface of the cell surface phase. This means that the rate of transfer in either direction is proportional to the

gradient, at a fixed extracellular glucose level; but inversely proportional to the extracellular concentration, with a fixed gradient; and that the inward rate is limited, while the outward rate may be much faster. This simple system gradually fails to operate as the glucose level in the medium is raised above about three-fourths isosmotic; this inhibition is reversible. An empirical correction is derived for a known but hitherto uncalibrated optical disturbance complicating the use of nonelectrolyte solutions in the Ørskov apparatus.

Prevention of development of cardiac arrhythmias by regitine (C-7337). ALFRED LEIMDORFER. *Dept. of Pharmacology, Loyola Univ., Chicago, Ill.*

Cardiac arrhythmias were produced in dogs under Nembutal anesthesia by the intravenous i.v. injection of epinephrine hydrochloride (0.004–0.007 mg/kg). Several minutes later, regitine was injected i.v. (0.25–0.33 mg/kg). After regitine injection, there was only a slight transitory fall in blood pressure (10–15 mm. Hg) lasting about 5 minutes, then the blood pressure returned to the preinjection level. The ECG's (after regitine) showed usually a tachycardia sometimes with reduction in the height of the R-waves, and sometimes a transitory depression of the S-T segments. When epinephrine was injected i.v. in the same amounts as previously, 5, 10, 15, 30, 45 and 60 minutes after regitine, no cardiac arrhythmias appeared. This preventive effect of regitine lasted usually about 1 hour. There was no blockade or reversal of the blood pressure if epinephrine was given following regitine, only a slight reduction in the blood pressure response took place in comparison to the blood pressure response to epinephrine without regitine. Sometimes there appeared a few singular P-waves or some beats of nodal rhythm after the epinephrine injection following regitine. This was considered as a shift of the pacemaker due to a vagal action of epinephrine. After the subsequent i.v. injection of atropine sulfate (0.1 mg/kg.) these ECG disturbances also disappeared. In this way regitine or the combined injection of regitine and atropine prevented the appearance of cardiac arrhythmias induced by epinephrine. In other experiments arrhythmias which are provoked by the injection of arterenol could be prevented also by the injections of regitine, only larger doses were needed in these cases.

Pathology of spinal fluid from hypertensive patients. ALFRED LEIMDORFER. *Dept. of Pharmacology, Loyola Univ., Chicago, Ill.*

In acute experiments on dogs (under Pentothal anesthesia), small amounts (2 cc.) of spinal fluid of hypertensive patients were injected intracisternally (i.c.) after removal of an equivalent volume of cerebrospinal fluid. Electrocardiograms (ECG) and the blood pressure were recorded continuously. Within 10–20 minutes after the injection of the spinal fluid, there developed a change in the ECG of the dogs, the previously upright T-waves in the second lead became gradually isoelectric and finally completely inverted. The intravenous (i.v.) injection of the spinal fluid of such patients also provoked similar changes in the ECG's of the dogs. Of interest might be the fact that the ECG of such patients showed inverted T-waves in all standard leads which was taken as indications of myocardial pathology of these patients. The blood pressure of the dogs was only slightly altered after the injection of these spinal fluids, perhaps due to the depressive action of the Pentothal anesthesia. In contrast the i.c. or i.v. injection of spinal fluid of normal persons was not followed by any change in the ECG or blood pressure of the dogs.

Thyroxin binding by bovine serum albumin. ALLEN LEIN. *Dept. of Physiology, Northwestern Univ. Med. School, Chicago, Ill.*

Recent evidence presented by Taurog and Chaikoff (*J. Biol. Chem.* 176: 639, 1948), Laidlaw (*Nature* 164: 927, 1949) and by Gross *et al.* (*Science* 111: 605, 1950) identifies thyroxin as the circulating form of the thyroid hormone. Since other work has long supported the view that the thyroid hormone circulates bound to serum protein, the characteristics of the binding of thyroxin by serum proteins are of considerable interest. The present study was thus undertaken in order to estimate the thyroxin binding capacity of serum albumin and to measure the strength of the protein-thyroxin bond formed. Crystalline bovine serum albumin (Armour and Co.) was employed and the binding of thyroxin to the protein determined at 25°C. by the method of equilibrium dialysis. Glycinate buffer (0.10 M at pH 9.75) was used, and thyroxin was measured spectrophotometrically at 330 mμ. Binding data were treated in accordance with procedures developed by Klotz, Scatchard, and by Karush. The data support the theory that bovine serum albumin at pH 9.75 possesses 2 species of binding sites for thyroxin. For one type of binding, the average maximum number of binding sites/molecule of protein, n_1, is approximately 4; the intrinsic association constant, K_1, = 7.9 x 19^4 and the free energy of binding, ΔF_1, = −6.62 kcal. For the second binding site, n_2 is approximately 8, $K_2 = 0.10 \times 10^4$, $\Delta F_2 = -4.05$ kcal. For concentrations of thyroxin lower than 0.5×10^{-4} M, the second type of binding is of little significance, and equations derived for a single binding constant then offer a good fit for the experimental data.

Observations on the genesis of the U wave of the electrocardiogram. E. LEPESCHKIN (introduced by W. RAAB). *Div. of Exptl. Medicine, Univ. of Vermont, Burlington.*

In normals, the area of the chest showing the highest U waves in unipolar leads usually coincides with the area showing greatest amplitude of the protodiastolic low-frequency vibrations corresponding to the third heart sound. The U wave always ends after the maximum of these vibrations and in many cases is synchronous with a proto-diastolic outward movement of the apex beat. It begins always after the beginning of the second heart sound. Exercise or epinephrine causes U to move onto the descending limb of the T wave; this is accompanied by a corresponding earlier beginning of the second heart sound. Digitalis conversely causes the U wave and the second heart sound to begin later with respect to the T wave. In the few cases where a U wave was present in leads from the exposed frog heart, it remained as a negative after-potential when the curve was made monoplasic by injury; in these cases it coincided with the descending branch of the mechanogram. These observations favor the conclusion that the proto-diastolic relaxation of the ventricular muscle plays a role in the genesis of the U wave.

Determination of body fat by cyclopropane absorption. GERSON LESSER,* ARNOLD BLUMBERG* AND J. MURRAY STEELE. *Research Service, Third (NYU) Med. Div., Goldwater Memorial Hosp., and New York Univ. College of Medicine, New York City.*

Total body fat of rats was determined *in vivo* by measurement of the quantity of cyclopropane absorbed by the animal at a known tension of the gas. A measured quantity of cyclopropane was administered to each animal in an enclosed gas chamber of known volume. The tissues of the rat were found to come into equilibrium with cyclopropane at the tension of the gas existing in the chamber in about 90–150 minutes. After this time had elapsed the concentration of cyclopropane in the chamber was determined by oxidation with iodine pentoxide. From the solubility coefficients of cyclopropane in fat and nonlipid tissues and the concentration of cyclopropane in the chamber, the fat content of the rat was calculated. Due to its extremely high fat:water solubility ratio (approximately 52:1), about 90% of all cyclopropane in the body is present in fat once equilibrium occurs. Since relatively small amounts of cyclopropane are present in nonlipid tissues, even large errors in estimation of the cyclopropane in the nonlipid tissues cause only minor errors in the body fat determination. Values for total body fat by this method were found to approximate closely the weight of total ether extractable material. Mean weight of fat of 10 white rats by cyclopropane absorption was 40.4 gm., by ether extraction 40.1 gm. The average deviation was ± 2.1 gm. Average fat content by both methods was 13.2% of body weight, with an average deviation of ± 0.7%.

Metabolic characteristics of the guinea pig seminal vesicle. H. A. LEVEY* AND C. M. SZEGO. *Dept. of Zoology, Univ. of California, Los Angeles.*

The present investigation concerning the normal *in vitro* metabolic pathways of the guinea pig seminal vesicle was undertaken to establish base lines for the subsequent study of the influence of hormonal treatment upon the characteristic metabolism of the tissues involved, with a view toward localization of the effects of testosterone. The Q_{O_2} of the glandular epithelium of the seminal vesicle is 9.39 ± 1.10 (n = 37); that of the smooth muscle is 1.95 ± 0.51 (n = 39). Therefore, it is necessary to study these component tissues separately in order to avoid masking effects in the intact gland. Seminal vesicle succinoxidase activity is low compared with liver or kidney (Q succinate epithelium = 25; muscle = 15) and is typically inhibited by malonate, although malonate is relatively ineffective in inhibiting the endogenous respiration of these tissues. Sixteen days after castration the Q_{O_2} of epithelium drops 50–60% with no accompanying decline in Q succinate. Thus, the succinoxidase system appears not to be the limiting factor in the aerobic metabolism of the epithelium after castration. The Q_{O_2} of the smooth muscle is not markedly depressed 16 days after orchidectomy. The effects of intravenous testosterone administration on these phenomena will be reported. Oxygen consumption is not stimulated by β-hydroxybutyrate and although β-hydroxybutyrate dehydrogenase occurs in epithelium and muscle in low concentrations, it is probably of little importance in normal metabolism. Aerobically, in the presence of glucose, the net gain of lactate in normal epithelium is 5 times that in muscle. (This investigation was aided in part by a grant from the Cancer Research Coordinating Committee, University of California.)

Co-contraction and reciprocal innervation in normal voluntary human movement: utilization of reciprocal innervation in therapy of spasticity in neuromuscular disease. MILTON GJELHAUG LEVINE AND HERMAN KABAT. *Kabat-Kaiser Inst., Vallejo, Calif.*

Reciprocal innervation and co-contraction play an important role in the understanding and therapy of neuromuscular diseases. The presently accepted concept that reciprocal innervation applies to normal voluntary human movement is an

oversimplification. Electromyographic findings are presented to indicate that in such activity co-contraction is common and reciprocal innervation as yet uudemonstrated. Experiments indicate, however, that reciprocal innervation is important in spasticity of muscles in neuromuscular disease, and evidence is presented for the utilization of Sherrington's phenomenon in the therapy of spasticity. Briefly, co-contraction is evident in antagonist muscles when resistance is applied to agonist contraction. It is also evident in muscles acting on more than one joint where at times the same muscle may act as an antagonist and as a synergist. Even in single joint muscles, co-contraction is evident when muscles have a multiple function. The biceps brachii will act in supination at the same time as the triceps extends the elbow. Co-contraction also occurs in all antagonist muscles in the shortened range of contraction of the agonist. In this case proprioceptive stimulation causes the stretched antagonist to begin to contract. It is true that in some simple relatively unresisted voluntary movements there is antagonist inactivity, but in the human it has not as yet been demonstrated that in these particular cases inhibition occurs. Reciprocal innervation has been utilized in the treatment of spasticity by stimulating electrically the muscle antagonistic to the spastic one, and at the same time causing the agonist to move through its complete range of motion. With proper technique the spastic agonist shows varying degrees of relaxation.

Effect of experimental venous obstruction on water and electrolyte distribution in man. MARVIN F. LEVITT, LOUIS B. TURNER AND AVRON Y. SWEET (introduced by CHESTER W. HAMPEL). *Depts. of Medicine and Pediatrics, Mt. Sinai Hosp., New York City.*

Local obstruction of venous return effects an acute reduction of plasma volume and a depletion of interstitial fluid proximal to the venous block. A study was made to determine whether intracellular fluid and electrolytes might be mobilized under such circumstances. Accordingly, venous obstruction was produced by tourniquets applied to the thighs and the acute effects of this procedure on total extracellular volume (inulin space) were investigated. Five normal fasting subjects were infused with inulin at a constant rate for 5 hours until maximum volume of distribution had been achieved. Thereafter blood pressure cuffs were applied to both thighs and maintained at subdiastolic pressures for 2½ hours without changing inulin infusion rate or providing exogenous salt or water. Inulin space, glomerular filtration rate, blood hematocrits, plasma protein and electrolyte concentrations were measured serially before and during the application of the tourniquets. After 2½ hours of diminished venous flow, each of the 5 patients manifested an increase in the volume of distribution of inulin of 9–27% (av. 17%) above control values. Blood pressure and glomerular filtration rate showed a variable reduction. Coincidentally slight increases in hematocrit and plasma protein concentration were noted but no consistent change occurred in serum electrolyte concentrations. These data suggest that diminished venous flow, either through local or systemic effects, induces an endogenous expansion of the isotonic extracellular volume with the salt and water derived from intracellular stores.

Effects of graded hypoxemia on the pulmonary circulation of the dog. II. Moderate hypoxemia. BENJAMIN M. LEWIS AND RICHARD GORLIN (introduced by GEORGE W. THORN). *Med. Dept., Peter Bent Brigham Hosp. and Harvard Med. School, Boston, Mass.*

Nine dogs anesthetized with morphine-chloralose-urethane were subjected to breathing mixtures containing 10% oxygen. Duration of hypoxia varied from 20 minutes to 8 hours during which 19 sets of observations were made. Arterial oxygen saturation varied from 55–79%. Pulmonary artery (PA) and left atrial (LA) pressures were recorded through indwelling catheters. Cardiac output (CO) was measured by Fick principle. Pulmonary vascular resistance (PVR) was calculated from the Poisseuille equation. CO did not vary significantly from control values regardless of the duration of hypoxemia. Oxygen consumption and arterio-venous oxygen difference did not change appreciably. PA pressure rose somewhat and LA pressure fell somewhat, so that the pulmonary pressure gradient increased. PVR showed a statistically significant increase. The degree of increase varied directly with the duration of hypoxemia. A dog exposed to hypoxia for 7 hours with a rise in PVR was then allowed to breathe room air for 3 hours and subsequent observations showed a fall in PVR. When hypoxia is mild, our experiments are similar to those of others who have observed pulmonary vasoconstriction. However, previous experiments of this group have shown when hypoxia is so severe that cardiac output must rise to maintain tissue oxygen supply, PVR does not rise. The former may be due to a local effect of hypoxia on the lung, while hypoxia of the body as a whole appears to reverse the pulmonary vasoconstriction.

Inhibition of fibrinolysin and trypsin. JESSICA H. LEWIS AND JOHN H. FERGUSON. *Dept. of Physiology, Univ. of North Carolina, Chapel Hill.*

Lima bean inhibitor (Tauber *et al.*) (LBI), pancreatic inhibitor (Pan I) and dog serum antifibrinolysin (antilysin) were tested for their action

on the proteolytic enzymes, trypsin and dog serum fibrinolysin. Enzyme activity was assayed by a fibrinolytic technique. On both trypsin and fibrinolysin LBI and Pan I produced their full inhibitory effects rapidly, while, only in very concentrated form did antilysin have any immediate inhibitory action. Trypsin activity was decreased in direct proportion to the added amounts of LBI or Pan I. Fibrinolysin was inhibited by LBI, in concentrations similar to those which inhibited trypsin, but in this case the inhibitory effects were not proportional to the quantities of inhibitor added but were found to follow closely the law of mass action for a reversible reaction between fibrinolysin and LBI. LBI was able to prevent in part the natural deterioration of fibrinolysin and also the inhibition of fibrinolysin by weak antilysin. This might suggest that the natural deterioration of fibrinolysin was due to contaminating antilysin even though this fibrinolysin was prepared from apparently antilysin-free precursor. LBI did not prevent the inhibition of trypsin by antilysin. Pan I inhibited fibrinolysin only weakly, and in moderately high concentrations a direct proportionality was demonstrated between the amounts of inhibitor added and the degree of inhibition. Antilysin inhibited trypsin somewhat more readily than fibrinolysin, and in both cases complete inhibition was obtained over a wide range of inhibitor concentrations, the rate of inhibition being dependent upon the quantity of antilysin present.

Facilitation at neuromuscular junctions by stretch of muscle. B. LIBET AND E. W. WRIGHT.* *Univ. of California Med. School and College of Dentistry, San Francisco.*

Stretch of frog muscle augments the muscle action potential response to supramaximal nerve shocks; apparently some muscle fibers respond in stretched but not in unstretched muscle (RALSTON *et. al., Am. J. Physiol. 159:* 586, 1949). Partial curarization makes this effect evident in monkey, indicating stretch acts at the neuromuscular junction (LIBET AND FEINSTEIN, *Am. J. Physiol.* Dec., 1951). Further investigation with the frog's sartorius shows that this effect of stretch on muscle action potentials (responding to supramaximal nerve shocks) is exaggerated by partial curarization; with almost full curarization, small action potential responses appear in stretched muscle when none are present in unstretched muscle. The *end-plate-potential* is approximately doubled in amplitude when a slack muscle is stretched with less than 1 gram; further stretch produces relatively small additional increases. Preliminary experiments indicate that acetylcholine in just threshold concentrations (10^{-7}–10^{-6}) produces similar responses in stretched and unstretched muscle; prostigmine (10^{-5}M) does not abolish the effect of stretch on single action potential responses, but, with stimuli at 25/second, a more rapid fall-off in amplitude occurs in stretched than in unstretched muscle. This may mean that facilitation of junctional transmission by stretch is not due to increased responsiveness of the endplate region of the muscle fiber, but rather to greater output or more effective localization of the 'transmitter'. (Contribution from the Biomechanics Group, supported by grants from the National Foundation for Infantile Paralysis, and the Prosthetic Devices Funds of the Veterans Administration).

Effect of sodium fluoroacetate on the metabolism of isotopic acetate and lactate by the isolated perfused dog gastrocnemius. NATHAN LIFSON AND JOYCE A. STOLEN.* *Dept. of Physiology, Univ. of Minnesota, Minneapolis.*

The isolated gastrocnemius of a nembutalized dog was perfused at physiological pressures and temperatures, by means of a closed pump-oxygenator circuit, the animal's own heparinized blood serving as the perfusion fluid. Supramaximal stimuli were applied to the sciatic nerve at a rate of 2/second. Isotopic acetate (carboxyl-labeled) or lactate (either carboxyl or alpha and beta labeled) was injected to give a concentration in the blood-muscle system of approximately 15 mM/l. The effects of sodium fluoroacetate are described for concentrations of approximately 1mM/l. administered at least 30 minutes before the substrate. The NaFA poisoned preparations were characterized, as compared with unpoisoned ones, by weaker contractions, a reduction in the rate of CO_2 production, a greater depletion of glycogen, a rise in blood lactate, and a fall in blood pH. The conversion to respiratory carbon dioxide of the carboxyl carbon of acetate and of the noncarboxyl carbons of lactate is drastically inhibited by NaFA, but the conversion of the carboxyl-carbon of lactate to respiratory CO_2 still proceeds at significant rates under these conditions.

New criteria for the division of the acoustic cortex into functional areas. JOHN C. LILLY AND RUTH CHERRY.* *E. R. Johnson Fndn., Univ. of Pennsylvania, Philadelphia.*

Rose and Woolsey (1949) divide the acoustic cortex of cats into acoustic I and II (AI and AII) and the posterior ectosylvian region (Ep). Part of their evidence was obtained from maps of the distribution of evoked potentials recorded with a single electrode moved over the cortex; they selected those regions showing the maximal amplitudes of the evoked potentials. We have recorded, with 25 electrodes simultaneously, click responses

and spontaneous activity in the auditory cortex (*Proc. Am. Physiol. Soc.* Sept. 1951). We have tested this division into 3 areas by 2 additional criteria: *1*) the averaged courses and the averaged speeds of movement of a small, single value of potential during the rising and the falling phase of the evoked response and during the passage of a spontaneous figure; and *2*) the shapes and the velocities of many equipotential lines during the 2 types of activity. We conclude that AI and AII can be separated clearly from one another and from Ep during the rising phase of a single response at any given point but not so clearly during the falling phase. Further, Ep can be separated from AII by recording the changes in shape and in velocity of a spontaneous figure moving from Ep into AII. Thus, the origins, speeds, preferred directions of travel, and forms of the figures in responses and spontaneous activity are additional criteria which validate this functional division of this region of the cat's cortex. (Supported in part by U.S.P.H.S., Division of Research Grants.)

Further observations on the adenteric reflex and gastric inhibitors. R. K. S. LIM, E. A. NOVAK* AND FRANK WALSH*. *Dept. of Physiology and Pharmacology, Creighton Univ., Omaha, Nebr.*

Observations on motility and secretion in dogs with pyloric pouches and fundus fistulae, have failed to show any constant relationship between gastric motility and the secretion of gastrin elicited by mechanical (distension) and chemical (irrigation with 10 ml. 0.4% acetylcholine chloride) stimulation of the pylorus. Although both methods of pyloric stimulation accelerate and augment pyloric rhythmicity, the motor effect is evanescent while the secretory response is maintained. The lack of correlation thus indicates that excitation of the gastrin secreting cells depends upon the state of tension or stretch in the pyloric mucosa or subjacent submucosa, rather than the contractile changes in the muscular wall. This is in accord with the concept that the mechanism of gastrin liberation involves an adenteric reflex. Several atropine-like agents, viz. 0.25–0.5% methantheline bromide, 0.16–0.3% SKF 1494 and 0.15–0.25% SKF 1637 applied topically to the pyloric mucosa for 30 minutes in 10 ml. volume, have manifested similar inhibitory effects on gastric motility and secretion as 0.05–0.1% atropine sulfate. As the pupil, salivation and heart rate were unaffected, the gastric inhibition must be attributed to a local blocking action. The fact that the duration of motor inhibition was generally much shorter than the period of secretory depression suggests that two separate neural mechanisms are concerned, the myenteric plexus with the motor and the adenteric with the secretory.

Selective ionic accumulation in muscle cells. GILBERT LING. *Wilmer Inst., Johns Hopkins Hosp., Baltimore, Md.*

It has been postulated that selective cationic accumulation is essentially a result of the differences in magnitude of coulombic forces exerted on the various cations of different hydrated diameters, by the fixed charges of the protein-ion network in the cell protoplasm (*Am. J. Physiol. 167:* 1951). This hypothesis was extended to offer a similar mechanism for selective anion accumulation and a possible explanation for the effective Na^+ 'impermeability' at the cell boundaries (in press). If muscle cells are bathed in equimolar concentrations of different alkali metal ions, the final equilibrium intracellular concentrations of these ions should, according to the present hypothesis, not be equal, but should vary inversely with their hydrated diameters. Values of hydrated diameters as illustrated by the Hofmeister series: $Li^+ > Na^+ > K^+ > Rb^+ > Cs^+$, were calculated mostly from conductivity data extrapolated to infinite dilution. In biological studies, however, the ionic strength (μ) is usually about 0.1 M. Using Gorin's equation and available conductivity data, the following values of hydrated diameters were obtained for $\mu = 0.1$ M. (18°C.): Rb^+, 1.58 Å; K^+, 2.78 Å; Cs^+, 3.20 Å; Na^+, 4.00 Å; Li^+, 5.08 Å. The most notable feature of these results is the altered position of Cs^+ in the series. When frog muscles were soaked in a Ringer's solution containing: $K^+ = 0.0$ mM., $Rb^+ = Cs^+ = Li^+ = 2.5$ mM., K^+ was lost from the muscles; it was replaced principally by the smallest Rb^+, to a lesser extent by Cs^+, and least by the largest Li^+. This quantitative relationship among the equilibrium concentrations of ions accumulated within the cells supports the present hypothesis.

Effect of burn shock on concentration of nonprotein sulfhydryl in mouse liver. WAYNE LINKENHEIMER* AND LYLE V. BECK. *Dept. of Physiology and Pharmacology, Univ. of Pittsburgh, School of Medicine, Pittsburgh, Pa.*

Each mouse liver was homogenized in a Waring blendor for 1 minute with 19 volumes of cold M/10 potassium phosphate buffer, pH about 6.8. Proteins were precipitated immediately in an aliquot of the homogenate by sulfosalicylic acid, to give a filtrate. Debris was removed from another aliquot of the homogenate by rapid centrifuging to give a supernatant. Sulfhydryl concentrations were determined in the filtrate and in the supernatant by the amperometric titration method of Weissman *et al.* (*J. Biol. Chem.* 187: 153, 1950). Total extracted sulfhydryl was calculated from the value for the supernatant, nonprotein (glutathione) sulfhydryl from the value for the filtrate, and protein sulfhydryl as the difference. About

¾–⅞ of the total sulfhydryl extracted from livers of control mice was nonprotein sulfhydryl. Protein sulfhydryl constituted about 6–9% by weight of the protein, as measured by the Biuret method. Nonprotein sulfhydryl concentrations were markedly lower in livers secured from mice 3 hours after they had been etherized and scalded to the neck at 60° or 65°C. for 10 or 15 seconds, than they were in livers from untreated mice, or from mice which had been etherized and dipped in water at 37°C. The percentage of total sulfhydryl present as nonprotein sulfhydryl was appreciably decreased by burn shock.

Distribution of I^{131}- and C^{14}- labeled thyroxine in rat liver cell fractions, and elimination by way of bile, urine, and expired air. HARRY J. LIPNER*, HOWARD M. KLITGAARD,* SAMUEL B. BARKER AND THEODORE WINNICK.* *Dept. of Physiology and Radiation Research Lab., State Univ. of Iowa, Iowa City.*

Liver fractions were prepared by a modification of Schneider's method. Following subcutaneous administration, thyroxine was found in highest concentration in the supernatant fraction (50–60% of the total in the homogenate). The quantities localized in the nuclear fraction and in the large granules (mitochondria) were approximately equal (17–30% of the total). There was no apparent difference in distribution between C^{14} and I^{131} in the cellular fractions. Fractionation of a liver homogenate after *in vitro* addition of I^{131}-thyroxine revealed a distribution similar to that *in vivo*. Further fractionation of the supernatant from the *in vitro* experiment indicated an almost equal distribution of thyroxine between small granules (microsomes) and the almost particle-free phase. The findings of Taurog and co-workers relative to thyroxine excretion in the bile, and its presence, in part, in an unknown form in bile, were confirmed. Bile samples from rats given C^{14}-thyroxine were treated with ninhydrin. Virtually all of the C^{14} was released as $C^{14}O_2$, indicating that both amino and carboxyl groups were uncombined in the unknown thyroxine derivative. About 10% of the C^{14} appeared in expired air, and only 2% in the urine in 24 hours. (Aided by grants from Baxter Laboratories and Hoffmann-LaRoche.)

Sex differences in pathogenesis of nephrotoxic globulin (NTG) nephritis. RICHARD W. LIPPMAN, HELEN U. MARTI* AND E. ELMO JACOBS.* *Inst. for Med. Research, Cedars of Lebanon Hosp., Los Angeles, Calif.*

A sex difference in proteinuria of the normal rat was first observed by Addis (*Proc. Calif. Acad. Med.* March 1931, July 1932) and later studied in detail by Sellers *et al.* (*Am. J. Physiol.* 163: 662, 1950). According to these workers, normal male adult rats of the Slonaker-Addis strain excrete 4 or 5 times more protein daily than female adults. In this experiment, rabbit anti-rat-kidney gamma globulin (NTG) was administered to the rat under standardized conditions. The rate of proteinuria was measured at weekly intervals up to 4 weeks. In the male, a maximum rate of 118 mg/24 hr/100 gm. body wt. was reached at the end of 1 week, and this declined to 33 mg/24 hr/100 gm. body wt. at the end of 4 weeks. In the female, a maximum rate of only 55 mg/24 hr/100 gm. body wt. was reached at the end of 2 weeks, and this declined to 15 mg/24 hr/100 gm. body wt. at the end of 4 weeks. The animals were then killed and the kidneys were sectioned for histologic examination. A general description of the renal lesions observed has been given in detail (*Arch. Path.*, in press), and included proliferative glomerulitis with the deposition of fibrinoid material in or between the capillary loops. In the female, the degree of damage observed was consistently and considerably greater than that which was observed in the male. The sex difference, in the response to NTG administration, must be considered in evaluation of previously reported work by other authors, some of whom have given results that are not segregated with respect to sex.

Effect of oxypolygelatin on hemoglobin excretion in rat. RICHARD W. LIPPMAN, HELEN U. MARTI* AND JEAN OLIVER. *Inst. for Med. Research, Cedars of Lebanon Hosp., Los Angeles, Calif., Hoagland Lab., Dept. of Pathology, College of Medicine, State Univ. Med. Center, New York City.*

Oxypolygelatin has been suggested by Campbell *et al.* (*Texas Rep. Biol. & Med.* 9: 235, 1951) as a practical plasma substitute. However, Addis, Oliver, and others, as a result of proteinuria, glomerular and tubular damage, and azotemia, which they observed after administration of the original preparations, concluded that oxypolygelatin is toxic in the rat. In order to determine the factors responsible for such toxicity, the mechanism of proteinuria after oxypolygelatin administration was studied in the rat by means of hemoglobin and creatinine clearances, as previously used to study other types of proteinuria (*J. Exper. Med.* 93: 325, 605, 1951). It was found that after oxypolygelatin administration the threshold for hemoglobin excretion was sharply reduced but the slope of a line relating hemoglobin excretion to the product of glomerular filtration rate and serum hemoglobin concentration was not significantly altered from control values. From this it was concluded that oxypolygelatin 'saturates' the tubular hemoglobin absorption capacity but does not affect glomerular permeability. Examination of the tissue sections stained with benzidine confirmed these conclusions. It may be

suggested that the proteinuria which follows oxypolygelatin administration in the rat is the result of tubular epithelial 'saturation' with small molecules that pass the normal glomerular membrane. Since the oxypolygelatins prepared to date are polydisperse systems, with a wide variation in molecular size from 10,000 up, depending upon the precise conditions of preparation, it may be possible to eliminate the proteinuria and the principal toxicity by fractionation to remove the molecules of small molecular size.

Effect of increasing right ventricular load on the isolated left ventricle. ROBERT C. LITTLE, A. O. FITZMORRIS* AND JOHN F. BLANKENSHIP.* *Dept. of Physiology, Univ. of Tennessee, Memphis.*

The circulation from the right and left heart was separated by means of a modified dog heart lung preparation. The pulmonary artery was cannulated in both directions: blood from the right ventricle filled a reservoir that emptied into the right atrium, blood from a second reservoir perfused the lungs and left side of the heart before returning to the reservoir via the carotid artery or aorta. The height of each reservoir was adjustable and the resistance to flow from each ventricle was controllable by a screw clamp. Right and left intraventricular and left atrial pressure were recorded optically and left ventricular output was measured with a recording rotometer. Left ventricular filling and outflow resistance remained constant. A sudden increase in right ventricular filling usually resulted in a slowing of the heart rate due to a second degree heart block. Prevention of this bradycardia by vagal section as suggested by Aviada *et al.* (*Am. J. Physiol.* 165: 261, 1951) did not occur. Coincident with the bradycardia left ventricular output increased while left ventricular diastolic pressure fell. These results indicate that left ventricular diastolic pressure and ventricular output may vary in opposite directions. This evidence supports the view that changes in diastolic stretch of one ventricle can occur without an increase in distending pressure. (Supported by a grant from the Natl. Heart Inst., U. S. Public Health Service.)

Electrocardiograms of small mammals. ELNA LOMBARD (introduced by J. W. REMINGTON). *Dept. of Physiology, Univ. of Wisconsin, Madison.*

Little literature is available on the normal electrocardiograms of small mammals. The purpose of this investigation was to study a series of records made on 7 different groups of mammals; the peromyscus, white mouse, wild mouse, guinea pig, spermophile, white rat and hamster. These records were taken on 3 different days for each of 10 animals/series and were analyzed for the electrical axis, manifest value, P-R interval, P voltage, QRS interval and R voltage. All records resembled the normal human ECG in basic pattern with the following exceptions: *1*) voltages were lower, *2*) intervals were shorter and *3*) the records of the smaller mammals showed no discernible T wave. Due to the latter exception, no comparison of either voltage or duration of the T wave was possible.

Conversion of C^{14}-butyrate to acetoacetate by heart muscle. VICTOR LORBER. *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

The conversion of β-C^{14}-butyrate to acetoacetate has been studied in the intact blood-perfused cat heart, and in slices of rabbit ventricle incubated in saline medium. Unlabeled acetoacetate, introduced initially, together with β-C^{14}-butyrate, was isolated at the close of the experiment by partition chromatography, and degraded to CO_2 and acetone by thermal decarboxylation. The acetone was isolated as the mercury complex. A part of the mercury-acetone complex was oxidized to CO_2, and the free acetone regenerated from the remainder was subjected to the iodoform reaction. Similar results were obtained in both the perfusion and slice experiments. The acetoacetate was found to be labeled, with the isotope restricted to the carbonyl and carboxyl positions. The isotope distribution ratio, CO:COOH, was 3 to 1. The results show that acetoacetate is formed from butyrate in cardiac muscle, and are consistent with a recoupling of two carbon units derived from butyrate as at least one pathway involved. The direct oxidation of butyrate to acetoacetate cannot be excluded as a participating mechanism.

Adsorption of dodecyl ammonium chloride by the human erythrocyte. WARNER E. LOVE (introduced by BRITTON CHANCE). *Dept. of Physiology, School of Medicine, Univ. of Pennsylvania, Philadelphia.*

The cationic surface active agent dodecyl ammonium chloride (DACl) is a potent hemolysin. This compound is hemolytic because, like the related anionic surface active agents, (*Biol. Bull.* 93: 202, 1947; *J. Cell. & Comp. Physiol.* 36: 133, 1950) it enormously increases the permeability of the erythrocyte to cations (*Biol. Bull.* 101: 200, 1951). In order to understand better the hemolytic action of DACl, its binding by the erythrocyte has been measured quantitatively. The adsorption of dodecyl ammonium ions by the cell is reversible. No orderly effect of temperature on binding has been found, but increasing the pH from 6.5–10 increases the cell-detergent affinity by a factor of about 100, probably because dodecyl

ammonium ions compete with hydrogen ions for the binding loci. Preliminary experiments indeed indicate that the surface of the erythrocyte binds hydrogen ions and thus support independently the postulated mechanism of binding. The relation between binding and hemolysis has been examined in the light of these data. From pH 6.5–pH 10 10^8 dodecyl ammonium ions bound/cell produce, within a factor of 3 or 4, a constant rate and amount of hemolysis, indicating the close relationship between hemolysis and the amount bound. This amount would be sufficient to cover one-third to one-fourth of the cell surface.

Determination of phenylindanedione. J. LOWENTHAL,* M. W. OGILVIE* AND L. B. JAQUES. *Dept. of Physiology, Univ. of Saskatchewan, Saskatoon, Canada.*

A method for the determination of phenylindanedione, a new anticoagulant, in plasma has been worked out. It is based on the extraction of phenylindanedione from acidified plasma by toluene, re-extraction into alkali and determination of the optical density at 338 mμ. One ml. plasma is diluted with 3 ml. distilled water, acidified with 1 ml. 2 N sulfuric acid and shaken mechanically with 15 ml. toluene in a glass stoppered test tube for 15 minutes. The 2 layers are separated by centrifugation and 10 ml. of the toluene layer are transferred to a clean test tube. Four ml. of 3 N sodium hydroxide are added, the mixture is shaken for 5 minutes, the 2 layers are separated and the optical density of the alkaline solution read at 338 mμ using the Beckman spectrophotometer. A standard has to be prepared by adding known amounts of phenylindanedione to plasma. Plasma concentration of phenylindanedione and prothrombin levels were determined in rabbits after oral and intravenous administration of a single dose. There is in both cases a period of latency for the hypoprothrombinemic effect. The latter is observed only after the anticoagulant can no longer be detected in the plasma. Peak plasma levels were found to be surprisingly low after oral administration. After continued oral administration the blood levels and prothrombin times reach a maximum value after 5 days.

Calibration of the nitrogen meter. NILS LUNDGREN,* CLAYTON S. WHITE* AND WALTER M. BOOTHBY. *Dept. of Respiratory Physiology, Lovelace Fndn. for Med. Education and Research, Albuquerque, N. Mex.*

The nitrogen meter gives an accurate indication of the concentration of nitrogen in a mixture of respiratory gases which may contain various amounts of water vapor when properly calibrated. If the gas is 'wet' drying is possible, but is undesirable for several reasons, one of which is an untoward effect on the speed of response. In using the meter for quantitative volumetric work calibration curves for mixtures containing various amounts of water vapor therefore are essential. Empirical data indicate that the difference between the 'wet' and 'dry' calibration curves cannot be explained by assuming a simple dilution of the nitrogen with water vapor. The nitrogen meter, like several of the other newer gas analyzers, does not 'automatically' take care of variation in the amount of water vapor present in the sample as do the older volumetric techniques such as the Haldane which yields a 'dry' result.

Plant physiological test for syphilis. DAVID I. MACHT. *Dept. of Pharmacology, Sinai Hosp., Baltimore, Md.*

The author has reported in numerous previous papers his experiments concerning the influence of blood sera in hydroponic solutions on growth of certain seedlings. In this way, studies were made on menotoxin and other toxic substances were discovered in the blood of pernicious anemia, trachoma, leprosy, pemphigus and certain psychoses. The blood of all bacterial infections and also of syphilis produced no phytotoxic effect on the root growth of Lupinus albus seedlings grown in plant physiological solutions under strictly controlled ecological conditions. Recent experiments led to the discovery of a new and characteristic property of syphilitic blood sera and spinal fluids when studied on specially prepared plants. While syphilitic sera and spinal fluids produced no inhibition in the root growth of normal Lupinus albus seedlings, they exert a very marked toxicity on the growth of vernalized or yarovized seedlings. This phytotoxicity is a quantitative one and runs parallel to the Wasserman and other serological tests. The toxic property of syphilitic serum appears to reside in its albumin fraction and not in the globulin fraction. The test may be useful for diagnostic purposes and is especially convenient for the evaluation, *in vitro*, of the anti-luetic efficacy of the various anti-biotics. Thus, penicillin, aureomycin, and terramycin neutralize the toxicity of syphilitic serum *in vitro*, while streptomycin and dehydostreptomycin produce no effect in this respect. There are only two other diseases which are phytotoxic for vernalized plants, but these can be easily differentiated by special biophysical methods.

Toxicity of *o*-phenanthroline for bacteria. ROBERT A. MACLEOD (introduced by M. E. SAWYER). *Dept. of Biochemistry, Queen's Univ., Kingston, Canada.*

The metal-binding agent *o*-phenanthroline, has been found to inhibit the growth of 8 lactic acid

bacteria studied. The inhibition produced by the addition of the chelating agent to the medium could be overcome by Fe^{++} and Ni^{++} and in some cases also by Co^{++} and Zn^{++}. In no case could the inhibition be reversed by ions implicated previously by more direct methods in the nutrition of the organisms. To learn more about the mechanism of the toxic action of *o*-phenanthroline the toxicity of the compound was compared with that of its *meta* and *para* isomers, compounds possessing similar structures but no metal-binding capacity. For *Streptococcus faecalis* R *o*-phenanthroline was found to be only slightly more toxic than its isomers in the absence of ions capable of overcoming its toxicity whereas the compound was much less toxic than its isomers in the presence of these ions. For *Lactobacillus arabinosus* and various other organisms the results obtained differed in that *o*-phenanthroline was 10–15 times as toxic as its isomers in the absence of reversing ions. The findings are consistent with the view that for *S. faecalis* the toxicity of *o*-phenanthroline is due to factors independent of its metal-binding capacity and that the action of various ions in overcoming its toxicity depends upon their ability to form nontoxic complexes with a toxic compound. For the other organisms, 2 mechanisms of action of *o*-phenenthroline appear to be operating, one which is dependent upon and one which is independent of, its metal-binding capacity.

Functional recovery following spinal cord hemisection and intrathecal use of hypochlorite treated trypsin. THOMAS P. MAGENIS,* L. W. FREEMAN AND DONALD E. BOWMAN.* *Indiana Univ. Med. Center, Indianapolis.*

A considerable degree of functional recovery was observed following hemisection of the spinal cords of dogs which received hypochlorite treated trypsin. Trypsin was used to diminish fibrin deposit in the belief that this would alter the mode and type of scar formation. Hemisection was chosen to insure continuity of fiber tracts as a control for function retained and function lost by tract section as it might be influenced by subsequent scarring. Hypochlorite treated trypsin was used because of characteristics described earlier (*Federation Proc.* 10: 165, 1951). The enzyme was administered by intrathecal instillations over a period of 6 weeks, the controls differing only in that saline was substituted for the enzyme. All animals received ACTH and cortisone intramuscularly. Functional return in the treated animals was in marked contrast to the progression to paraplegia in the controls. Tracking in the treated animals was apparent in walking, climbing, running, and jumping exercises. This was not true in the controls, and sensory differences were demonstrated. Gross inspection of the injured cords showed diminution of scarring and less constriction in the treated animals. Histological studies are in progress.

Microanalysis of fatty acid mixtures. SHU-HUAI MAI AND FRANK A. RAILA (introduced by K. K. JONES). *Dept. of Biochemistry, Northwestern Univ. Med. School, Chicago, Ill.*

A photographic method has been developed for the identification and quantitative determination of the straight chain even numbered fatty acids from C_{10} to C_{18}. The fatty acid dissolved in Skellysolve B is spread on aqueous solutions of different pH in a film of piston oil of known pressure. The area of the film is photographed by direct exposure of insensitive photographic paper placed under the tray. A series of pictures is taken for each spreading experiment at definite time intervals. Two characteristic properties of the fatty acids are observed: *1*), the area of spread, and *2*), the area of shrinkage of the film. The logarithm of the area of the acid spread is a linear function of time (from ½ to 3 minutes) at the pH where it shrinks. The rate of shrinkage is inversely proportional to hydrogen ion concentration. It has been found possible to find pH values at which a particular fatty acid disappears almost completely from the surface while all higher members of the series show negligible shrinkage after 5 minutes, the areas occupied by these acids being additive. By step-wise increase in pH one fatty acid after another can therefore be eliminated and the total composition of the mixture determined. Both spread and shrinkage can be used for the identification and quantitative determination of the fatty acids alone or in mixtures. The working range of the method is 0.2–5 μg. of each acid in the solution applied to the oil film. (Work partially supported by a U.S.P.H. Grant.)

Action potentials of motor cortex evoked by peripheral nerve stimulation. LEONARD I. MALIS,* KARL H. PRIBRAM AND LAWRENCE KRUGER.* *Lab. of Physiology, Yale Univ. School of Medicine, New Haven, and Burlingame Research Lab., Inst. of Living, Hartford, Conn.*

This report is the first of a series of studies dealing with the origin and course of afferents to the primate precentral cortex. With the experimental animals under barbiturate anesthesia single square pulse electrical stimuli were applied to various peripheral nerves. Recordings were made of the evoked cortical action potentials. The contralateral responsive area was mapped; it included both the postcentral and precentral gyri. Postcentral and precentral responses were of the same magnitude (300–500 microvolts) and the same general wave form. Postcentrally the latency

(measured to the maximum response) varied from 12–22 msec., depending upon the depth of anesthesia. Precentrally the latency increased with the distance anterior to the central fissure, reaching a maximum of 4 or 6 msec. longer than the postcentral latency. Stimulation of isolated skin or muscle nerves gave identical results. Acute ablation of the postcentral gyri bilaterally in combination with the ipsilateral precentral gyrus and the entire cerebellum did not alter the response in the remaining precentral gyrus. The extent of the surgical ablations was confirmed by serial reconstruction of the brains of the operated animals. These experiments suggest the existence of an afferent neural system from the periphery to the precentral cortex which does not include the postcentral cortex or cerebellum. (Supported in part by grants from the Abrahamson Foundation and the research fund of Mount Sinai Hospital, New York.)

Importance of the negative pressure phase in mechanical breathing machines. J. V. Maloney, Jr., J. O. Elam,* S. W. Handford,* G. A. Balla,* D. W. Eastwood* and R. H. Ten Pas.* *Naval Med. Field Research Lab., Camp Lejeune, and Div. of Anesthesiology, Washington Univ., St. Louis, Mo.*

The relative physiologic merits of the intermittent positive *vs.* the alternating positive-negative type mechanical respirators have been a subject of controversy for many years. The present experiments were designed to evaluate a wide variety of mechanical breathing machines under two clinical conditions: *a*) the relatively normal, lightly anesthetized individual, *b*) the individual with some form of respiratory and/or circulatory failure. Patients were studied in the operating room during routine surgical procedures, or on the hospital wards during treatment for respiratory failure. Physiologic measurements included the continuous and simultaneous recording on a direct writing multichannel oscillograph of arterial blood pressure, airway pressure, and cardiac output by the blue dye method. Ventilation was monitored with a ventigrator, or by a continuous analysis of alveolar air with a direct writing infrared carbon dioxide analyzer. Patients with a normal circulatory and respiratory system tolerated either the intermittent positive or the positive-negative pressure without difficulty. In these patients intermittent positive pressure caused no appreciable change in blood pressure, but an 11% fall in cardiac output. The effects of the respirators in patients with respiratory paralysis and/or circulatory inadequacy were quite different. Intermittent positive pressure produced a fall in blood pressure and an average decrease in cardiac output of 42%. Circulatory depression occurred regardless of the care used in administering the intermittent positive pressure. The degree of depression was not correlated with the pressure wave form, but appeared related to the mean airway pressure. Under the same conditions, the positive-negative type respirators had a salutary effect on the blood pressure and cardiac output. The disparity between the results in relatively normal patients and in those with respiratory and/or circulatory failure indicates that mechanical breathing machines are best evaluated in those emergency situations in which they are likely to be used.

Absorption of water, urea, glucose and electrolytes through the human bladder. N. S. R. Maluf. *Squier Urological Clinic, Columbia-Presbyterian Hosp., New York City.*

An approximately ideal occurrence permitted this study. A patient from whose bladder a small recurrent papilloma had been fulgurated a few weeks previously had undergone a bilateral uretero-sigmoidostomy in preparation for cystectomy. His bladder was essentially normal except for a small area of incipient intra-epithelial carcinoma which did not extend into submucosa. Solutions of accurately measured volume were introduced into the bladder through an indwelling urethral whistle-tip catheter taped in place so that its orifices were at the vesical neck. The catheter was connected with a mercurial manometer by narrow polyethylene tubing. The solution was introduced into the bladder through a small y-tube. There was no net diffusion of Na^+, Cl^-, and water through the bladder from an isotonic solution of NaCl. Na^+ and Cl^- diffused appreciably through the bladder from moderately hypertonic, 2% NaCl. Water simultaneously diffused into the bladder. There was no significant net diffusion of K^+ and Cl^- through the bladder from moderately hypertonic 2% KCl. Urea diffused appreciably through the human bladder. From 50 cc. of 2% urea in the bladder with a capacity of 200 cc., the hourly rate of diffusion was 56 mg. Glucose did not diffuse appreciably through the bladder in either direction. To test diffusion of glucose into the bladder the patient's plasma glucose was raised to 360 mg.% by intravenous drip of glucose, and isotonic NaCl was introduced into the bladder. Traces of Na^+, K^+, and Cl^- diffused into the bladder when the intravesical fluid totally lacked these. Distilled water introduced into the bladder diffused through the same.

Evaluation of methods measuring creatinine. Emanuel E. Mandel and Florence L. Jones (introduced by John L. Patterson, Jr.). *Communicable Disease Center, Public Health Service, Federal Security Agency and Dept. of Pathology, Emory Univ. School of Medicine, Atlanta, Ga.*

Recent modifications of the Jaffe (alkaline picrate) reaction and the Bolliger (3,5-dinitrobenzoate) reaction were studied as applied to 3 common types of serum filtrates and to urine, both with and without extraction of creatinine by Lloyd's reagent. Tungstic acid and cadmium hydroxide filtrates gave incomplete recoveries and yielded creatinine values 5–11% lower than corresponding trichloroacetic acid filtrates. When applied to Lloyd's reagent extracts of trichloroacetic acid filtrates (HARE. *Proc. Soc. Exper. Biol. & Med.* 74: 148, 1950), alkaline picrate furnished what appeared to be true values for creatinine; whereas dinitrobenzoate (ROETTGER. *Biochem. Ztschr.* 319: 359, 1949, modified), claimed to be more 'specific' than picrate, gave somewhat higher values. Both picrate (*J. Biol. Chem.* 158: 581, 1945) and dinitrobenzoate, when directly applied to filtrates, gave values which, at low serum creatinine levels, included up to 50% of noncreatinine chromogen; this fraction was smaller in azotemic sera, usually less than 10%. Results obtained in urine with either picrate or dinitrobenzoate were practically identical, irrespective of the use of Lloyd's reagent. Using Hare's picrate method in trichloroacetic acid filtrates, the method of choice for exact measurements, the serum creatinine content of venous arm blood was found to decrease slightly after clenching and releasing the fist for several minutes; there was no significant difference between venous and arterial blood.

Tension curves characteristic of muscles in which the fibers are not arranged in parallel. J. E. MARKEE AND M. WILLIAMS.* *Dept. of Anatomy, Duke Univ., Durham, N. C.*

By means of a strain gauge, we have recorded the total and the resting tension of certain muscles of the lower leg of the dog throughout the entire range of shortening of the muscle. After one or more of the nerve branches are cut or after parts of the muscle are cut, the peak of the active or the total tension curve is lowered but the shape of the curve is not altered in those muscles in which the muscle fibers are arranged in parallel. However, either of the above procedures changes the shape of the tension curves of those muscles in which the muscle fibers are arranged in series. By means of dissection it has been demonstrated that in most of the muscles of the lower leg of the dog the muscle fibers are arranged in parallel. In such muscles, destruction of part of the motor nerve or the muscle lowers the active tension curve but does not change the location of the peak of the active tension curve. On the other hand, the anterior tibial muscle, which is made up of five areas with separate innervations and which can be made to contract separately, contracts as though some of the five areas are arranged in series rather than in parallel. That is after destruction of any of the 5 motor nerve branches, the location of the peak of the active tension curve is changed.

Effect of 'thyroidectomy' on nephrectomized dogs and rats. ROBERT T. MARSHALL* AND SMITH FREEMAN. *Dept. of Biochemistry, Northwestern Univ. Med. School, Chicago, Ill.*

A study was made of the effect of 'thyroidectomy' upon the survival time and blood chemical changes of bilaterally nephrectomized dogs and rats. Destruction of the thyroid gland was accomplished by the administration of I^{131}. The mean survival time of 12 control dogs was 104.9 hours (S. D., 24.8), while that of 9 'thyroidectomized' dogs was 182.8 hours (S. D., 47.8). The difference between control and experimental groups is statistically significant (t ratio, 4.86). The serum concentration of inorganic phosphorus of the thyroidectomized group increased more slowly following nephrectomy than did that of the control group. There was no clearly defined difference between the 2 groups in the rate of increase of the serum potassium or N.P.N. The terminal values for serum potassium, inorganic phosphorus, and N.P.N. were all considerably higher in the thyroidectomized group. Similar studies were performed on adult rats of the Sprague-Dawley strain. The average survival time of 52 animals in the control group was 88.13 hours (S. D., 15.23) and that of 48 thyroidectomized rats, 107.72 hours (S. D., 17.0). The difference in survival time is statistically significant (t ratio, 6.07). Seventy-two hours after nephrectomy the concentration of serum inorganic phosphorus was definitely lower in the thyroidectomized rats.

Ethanol oxidation by rat liver slices. E. J. MASORO AND J. R. BIRCHARD (introduced by G. H. ETTINGER). *Dept. of Physiology, Queen's Univ., Kingston, Ontario, Canada.*

The effect that the concentration of ethyl alcohol has on the ability of the liver to convert C^{14}-labelled ethanol to $C^{14}O_2$ was investigated. It was felt that this basic information was needed before a study of other factors affecting ethanol metabolism could be effectively undertaken. The liver slices were incubated for 3 hours at 37.5° C. in 5 ml. of the Krebs-Henseleit bicarbonate buffer medium containing the following concentrations of C^{14}-labelled ethanol per milliliter of solution: 0.065 mg., 0.13 mg., 0.26 mg., 0.61 mg., 1.2 mg., 2.5 mg. Although there was considerable variation, from rat to rat, in the absolute number of milligrams and in the percentage of the added ethanol converted to CO_2, the curves obtained were of a similar form for each animal studied. When expressed in terms of milligrams of ethanol converted to CO_2, a curve was found with a peak at

the 0.26 mg/ml. concentration; the curve fell off abruptly at the 0.61 mg/ml. concentration, and was a plateau from this point on through the higher concentration range. When expressed in terms of percentage of the ethanol converted to CO_2, there was a rather gradual fall from the 0.065 mg/ml. to the 0.25 mg/ml. concentrations, and a much steeper fall from the 0.25 mg/ml. to the 2.5 mg/ml. concentrations. The physiological significance of the above data is now being investigated.

Toxicity of desoxycorticosterone acetate (DCA) in rabbits on high salt diet. G. M. C. MASSON, L. A. LEWIS, A. C. CORCORAN AND I. H. PAGE. *Cleveland Clinic Fndn. and Frank E. Bunts Educational Inst., Cleveland, Ohio.*

Thirty-two adult rabbits given 1% NaCl solution as drinking water were divided into 2 groups: animals of *group I* (21) received daily subcutaneous injections of 10 mg. of DCA as a water suspension, those of *group II* (11) kept as controls, received daily injections of the suspending medium (saline, alcohol, detergent). Some of the rabbits were mated prior to the beginning of the experiment. The following observations were made: *1*) Paralysis or clonic seizures appeared as early as the 5th day of treatment; *2*) interruption of pregnancy with fetal retention; *3*) normal blood pressure; *4*) normal hemoglobin, hematocrit, plasma chlorides and sodium values, but decrease in plasma potassium. *5*) High plasma cholesterol values (104 compared with 35 mg.% in controls) were associated with visible lipemia; ultracentrifuge studies using a density of 1.21 showed the presence of −S1.21 (>70) and −S1.21 (40–70) components in high concentration. The same components are not normally present in appreciable amounts. The −S1.21 (30–40) was slightly higher, while the −S1.21 (1–10) component was slightly lower than normal. *6*) Electrophoretic studies using phosphate buffer pH 7.8, μ 0.16, showed 2 α-globulin components (α_1 and α_2). In normal rabbits, only α_2-globulin is resolved and not always completely. The β-globulin was more concentrated than normal while the albumin and γ-globulin were decreased. *7*) The most significant pathologic change consisted of hepatic necrosis and subcapsular hemorrhages restricted to the right lobe. Since most of these symptoms as well as the chemical and pathologic changes seen in these rabbits are reminiscent of those seen in human eclamptogenic toxemia, it is suggested that salt-active corticosteroids may play a role in the pathogenesis of this condition.

Instantaneous equipotential data during the T interval on the body surface of the normal adult human. A. MAURO,* L. H. NAHUM AND RAJINDER S. SIKAND.* *Lab. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.*

The instantaneous potential distribution was plotted in terms of equipotential lines for 3 instants of the T interval. The data have been processed in the same way as in the earlier communication concerning the Q.R.S. interval (*J. Applied Physiol.* 3: 454, 1951) The striking difference in the potential distribution can be seen by the fact that the left side of the chest remains positive with respect to the right side. Moreover, the potential values merely wax and wane in time with a minimum of rotation. This is in sharp contrast to the sweeping rotation of the potential pattern over the surface during the Q R.S. interval. Although the distribution is a '2-pole function,' its irregularity and asymmetry make it impossible, as in the case of the Q.R.S. events, to invoke a vector or axis parameter to describe the *total* distribution from moment to moment.

Relation between oxygen consumption and wing load in flies. FRANCES V. MCCANN* AND EDWARD G. BOETTIGER. *Dept. of Zoology, Univ. of Connecticut, Storrs.*

The oxygen consumption of flies with different wing loads was determined by the Warburg method. The resting fly (Sarcophaga bullata, Parker), consumed an average of 28.7 mm^3/gm/min. With the wings removed to reduce muscle load to a minimum, the rate averaged 456 mm^3/gm/min., an increase of 16 times over the resting rate. As flight movement frequencies were also made, the results could be calculated in terms of oxygen utilization per cycle; this averaged $2 \times 10^{-3} mm^3$/cycle. The flight muscles of the wingless fly do work in deforming the elastic thorax. Although other evidence indicates that this is by no means small, it can be considered fairly constant throughout each cycle irrespective of wing load. With the wings on, the oxygen consumption was 725 mm^3/gm/min., or $7 \times 10^{-3} mm^3$/cycle. The increase in oxygen consumption per cycle observed must be related to the work done in moving the wings. If the oxidative processes are considered to be 50% efficient as has been found for frog muscle, half of the energy equivalent of the extra oxygen consumed is utilized in moving the wings. Assuming an R.Q. of 1, a rough calculation of the work done per cycle in moving the wings can be made. This calculates to be 100 ergs/cycle, or 18,000 ergs/second. Studies of flies with various wing loads were made to determine the relation between the extra oxygen used and the load.

Effectiveness of NaCl solutions in combating shock and promoting 10-day survival of severely burned rats. MILES D. MCCARTHY AND NANCY NEWLIN (introduced by I. S. RAVDIN). *Harrison*

Dept. of Surgical Research, Schools of Medicine, Univ. of Pennsylvania, Philadelphia.

A back burn at 90° C. for 35 seconds which is lethal to all untreated control rats (McCARTHY, *J. Lab. & Clin. Med.* 30: 1027, 1945) has been used to define the limits of effectiveness of NaCl infusions in combating shock and promoting 10-day survival. The severity of such burns was intensified by increasing the surface area burned from 32 ± 2% to 42 ± 2 and 50 ± 2% following hypertonic NaCl (1.4%) infusions amounting to 15% of the body weight (found to be the optimum infusion volume) survival percentages at 10 days were 75, 78 and 20, respectively. Furthermore, the effectiveness of the infusions was conditioned by the time lapse from burning to the beginning of therapy; e.g. the majority of rats receiving a 42% burn died when infusions were begun at 2 hours post-burn, but had a higher survival percentage when infusions were begun at one-half hour post-burn. (This work was supported in part by contract number DA-49-007-MD-107 between the University of Pennsylvania and the Department of the Army.)

Mineral metabolism of rats receiving sulfonic ion exchange resin chronically. EVAN W. McCHESNEY. *Sterling-Winthrop Research Inst., Rensselaer, N. Y.*

Rats have been kept on a diet of medium Na and K content for 9 months. Some have received ammonium sulfonate resin at levels of 5, 10, or 15%, and in one group the resin fed contained 1 mEq. of K/gm. There were only small differences in rate of growth of rats of the same sex, except at the 15% dietary level; these animals were only able to maintain their original weights. Data on the mineral metabolism of the female rats have been obtained at several intervals, and will be presented in detail. In general, the animals show borderline Na, K, and Mg while balances of Ca decrease as the rate of growth decreases. The resins cause a marked shift of Na and K from urine to feces but do not affect Ca or Mg metabolism appreciably. Phosphate absorption is markedly increased by the resin, while iron balances in both resin and control animals are near zero. The most significant feature of the results is the demonstration that in some groups practically 100% of the dietary Na and K appears in the feces.

An accelerator of prothrombin conversion arising in purified prothrombin preparations. ROBERT I. McCLAUGHRY* AND WALTER H. SEEGERS. *Dept. of Physiology and Pharmacology, Wayne Univ. College of Medicine, Detroit, Mich.*

Purified prothrombin prepared by the method of Ware and Seegers (*J. Biol. Chem.* 174: 565, 1948), when heated to 53° C. for 2 hours, is free from Ac-globulin. Such preparations may be dried by precipitation with acetone, and they remain stable for many months in the dried state. When dissolved in water or physiological saline solution and stored in a deep freeze, however, the prothrombin concentration progressively decreases over a period of 4–12 weeks. With the decrease in prothrombin activity, there becomes detectable in the purified prothrombin solutions an accelerator of prothrombin activation. This accelerator causes as much as a tenfold increase in the apparent Ac-globulin content of bovine plasma, as determined by the method described by Ware and Seegers (*J. Biol. Chem.* 172: 699, 1948). The accelerator effect could not be demonstrated by attempting to substitute it for thromboplastin or Ac-globulin in the activator analysis system; it seems rather to supplement these previously recognized activators. The accelerator is probably a derivative of prothrombin itself. The clotting time of fibrinogen solutions with standard thrombin solutions was not affected by the concentration of the prothrombin solution used in demonstrating the accelerator effect, although higher concentrations did cause an equivocal shortening of the clotting time.

Nucleic acid content of rat testes at various ages. WILLIAM B. McENERY* AND WARREN O. NELSON. *College of Medicine, Univ. of Iowa, Iowa City.*

Pentose (PNAP) and desoxypentose (DPNAP) nucleic acid phosphorus were determined by Schneider's modification of Schmidt and Thannhauser's method. Cytoplasmic basophilia was studied by the methyl green pyronin technique and desoxypentose nucleic acid visualized by the Feulgen procedure. PNAP values in mg/100 gm. fresh tissue in normal adult, and at 10, 20, 30, 40 and 60 days were, respectively, 60.32, 120.01, 110.84, 86.63, 70.31 and 59.45. DPNAP values were, respectively, 12.30, 25.81, 26.67, 20.83, 13.86 and 12.21. Cytochemical preparations of normal adult testes revealed considerable cytoplasmic basophila, presumably pentose nucleic acid, in the outer layer of tubular cells, spermatogonia and Sertoli cells and large extracellular basophilic droplets in the region of sperm maturation. Other cellular elements in normal adult testes showed moderate amounts of cytoplasmic basophilia, apparently proportional to the cytoplasmic content. Feulgen preparations indicated that nuclei of spermatogonia and primary spermatocytes were exceedingly rich in DPNA. Sperm heads showed considerable amounts, but spermatid and Leydig cell nuclei relatively little. In the 10- and 20-day testes the tubular cells showed increased cytoplasmic basophilia. This difference was not seen in older testes. Thus it has been concluded that

over and above the differences in cell types there is an increased amount of PNA. Since the Feulgen reaction was not appreciably different in any of the groups, differences seen in the DPNA values are attributable to the cell types present at the different ages. (This study supported in part by a research grant from the U. S. Public Health Service, Rg 1778.)

Nucleic acid content of rat testes following various experimental procedures. WILLIAM B. MCENERY* AND WARREN O. NELSON. *College of Medicine, State Univ. of Iowa, Iowa City.*

Chemical determinations and cytochemical visualization of pentose (PNAP) and desoxypentose (DPNAP) nucleic acid phosphorus were made (see McEnery and Nelson, page 103) on testes of rats subjected to several experimental procedures. PNAP values in mg/100 gm. fresh tissue in adult rats and after hypophysectomy (30 days), estrogen (100 μg. for 30 days), x-ray (30 days following 600 r) and cryptorchid (80 days) were, respectively, 60.32, 87.14, 86.01, 67.66 and 66.52. DPNAP values were, respectively, 12.30, 23.45, 23.24, 14.63 and 17.32. All these procedures impair spermatogenesis, however, the extent of impairment varies with the procedure and the tubular cell populations being correspondingly altered. A considerable increase in PNAP occurred after hypophysectomy and estrogen since the tubules contain Sertoli cells, spermatogonia and primary spermatocytes, all of which have low nuclear-cytoplasmic ratios. After x-irradiation and cryptorchidism, Sertoli cells predominate and while they have a low nuclear-cytoplasmic ratio apparently the PNA content is less than in other cells. DPNAP values were doubled after hypophysectomy and estrogen since all tubular cells have diploid numbers of chromosomes. We cannot explain why this was not true in cryptorchid and x-irradiated animals. Apparently these treatments produce some specific effect which is not understood. Cytochemical preparations of the experimental groups showed no measurable differences for the cell types present in each series. Thus it would appear that variations in nucleic acid content are due to the cell types present within the tubules. (This study supported in part by a research grant from the U. S. Public Health Service, Rg 1778.)

Tension-length curves of insect fibrillar muscle. WILLIAM MCENROE (introduced by ROBERT H. OSTER). *Dept. of Zoology, Univ. of Connecticut, Storrs.*

The fibrillar muscle of flies furnishes the power for their high frequency wing movements. This muscle contracts at a rate determined by the wing load. As soon as one stroke is finished the reverse stroke starts independently of CNS control. A fibrillar muscle therefore behaves as a fully tetanized muscle whose shortening is determined by the mechanical features of wing movement. Experiments were performed on large Sarcophaga flies to determine whether the fibrillar muscle, when prevented from shortening, will produce a constant tension. A technique was developed by which single muscles could be freed at one end and their tension recorded during tethered flight. With the muscle slightly stretched tensions of 250 mg. were obtained. The tension increased rapidly at the start of flight, was maintained constant during flight, and slowly disappeared at the end of flight. Tensions were also studied while stimulating the ganglion. The significance of these results in relation to theories of the operation of the fast flight mechanism of insects is discussed.

Histamine release from sensitized rabbit blood cells. Further evidence against the participation of plasma protease. FLOYD C. MCINTIRE* AND L. W. ROTH. *Abbott Labs., North Chicago, Ill.*

The theory that the development of anaphylaxis depends upon the activation of plasma protease has numerous proponents although this theory lacks the support of unequivocal evidence. In our studies of the early steps in the mechanism of the anaphylaxis reaction we have utilized the *in vitro* release of histamine from the blood cells and formed elements following the addition of antigen to the blood from sensitized rabbits. In a previous publication (MCINTIRE, F. C., L. W. ROTH AND M. SPROULL. *Proc. Soc. Exper. Biol. & Med.* 72: 605, 1950) we indicated that the participation of plasma protease in the *in vitro* histamine release was very doubtful because: *1*) excessive quantities of soybean trypsin inhibitor failed to inhibit the histamine release, and *2*) large amounts of bovine fibrinolysin failed to release significant amounts of histamine. As more conclusive evidence against the essentiality of plasma protease for the histamine release by antigen, we found that negligible histamine was released when rabbit plasma protease was activated by streptokinase (Lederle) sufficiently to lyse the clotted plasma in a few minutes. In the same rabbit blood the antigen-antibody reaction produced a marked release of histamine and did not activate enough protease to cause detectable lysis of the plasma clot. Furthermore, *in vivo*, no gross symptoms of anaphylaxis were produced by streptokinase sufficient to activate markedly the protease in 120 ml. of blood.

Studies of brain stem pressor responses in normal and hypertensive dogs. J. D. MCQUEEN,* AND A. EARL WALKER. *Div. of Neurological Surgery, Johns Hopkins Hosp., Baltimore, Md.*

The brain stem was explored in curarized dogs

by stimulation with thyratron discharges through a concentric electrode. Continuous blood pressure recordings were made from the aorta using an electromanometer. The electrical activity from the cortex and the points of stimulation was sampled; specific activation or after-discharge did not accompany the pressor responses. The cardiac slowing and irregularities which accompanied pressor responses were more easily suppressed with small doses of pentobarbital or ether than the rises in blood pressure. The latter were lost with a dosage of either of these agents sufficiently large to immobilize the animal. D-tubocurarine suppressed the pressor effects minimally up to a period of 3 minutes; sometimes not at all. Tetraethylammonium chloride, penta and hexa-methylene-1, 10-bis-trimethylammonium bromides abolished the pressor slowing and irregularities, lowered the level of blood pressure and suppressed the pressor responses. The 2 methonium derivatives were more potent in pressor suppression than tetraethylammonium chloride which was occasionally without effect. Epinephrine exerted its full influence in the presence of all 3. The decamethonium derivative did not exhibit these effects. The pressor responses found in hypertensive animals were essentially the same as those in normotensive animals. The significance of these results will be discussed.

A comparison of methods for measuring pulmonary resistance. JERE MEAD* (introduced by W. H. FORBES). *Dept. of Physiology, Harvard School of Public Health, Boston, Mass.*

A technique is described for the measurement of lung resistance in spontaneously breathing animals and man which utilizes a cathode ray oscilloscope as a coordinate plotting device. The plotted variables are: *1*) instantaneous rates of air flow on the 'Y' axis as obtained by means of a pneumotachograph screen and *2*) the resistive component of the pressure drop from the mouth to the surface of the lungs on the 'X' axis. The second variable is obtained by taking the pressure variation recorded from a latex balloon placed in the esophagus as an index of interpleural pressure variation and in effect subtracting the pressure component relating to lung elasticity by utilizing the linear relationship of this component to changing lung volume, which in turn is obtained by continuous integration of the rate of flow variable. The resulting pattern is a continuous plot of lung resistance with inspiratory and expiratory resistances recorded separately. Expressed in terms of the pressure in centimeters of water associated with a rate of flow of 100 l/minute, the range encountered in 7 normal adult subjects was 2.0–4.25 with a mean of 3.2. There was no significant difference between the phases of respiration. Lung resistance was substantially altered by changing mean lung volume, showing approximately a fivefold variation over the vital capacity range. Resistance measurements obtained by the interrupter technique in general were slightly higher than those obtained by the esophageal balloon technique. This finding is discussed in the light of theoretical considerations suggesting that the interrupter measurement includes tissue resistance of the lung and some component of thorax wall in addition to airway resistance.

Relation of diet to growth adaptation of young rats given large doses of hormones. JOSEPH MEITES. *Dept. of Physiology and Pharmacology, Michigan State College, East Lansing.*

The initial degree of growth-inhibition induced in young rats by administering large daily doses of hormones such as diethylstilbestrol, thyroid or cortisone is usually not maintained indefinitely. When the rat becomes adapted to the hormone overdosage, there is a distinct upward trend in growth which may or may not reach the level of untreated rats. Recent experimental data from this laboratory indicate that on an adequate diet, the initial inhibition of growth is accompanied by a decrease while the subsequent upward trend is accompanied by an increase in food consumption. On a deficient diet, the initial depressions of growth and appetite are more severe than on an adequate diet, and even after relatively long periods there is no marked upward trend in growth or appetite. These findings indicate that at least part of the ability of young rats to adapt themselves to overdosage with hormones depends on an increased intake of certain essential nutrients, i.e. vitamin B12, which are available in the adequate but not in the deficient diet. These observations are believed to constitute further evidence for the author's hypothesis that marked changes in the endocrine balance of the body decisively alter its nutritional requirements (*Metabolism*, Jan. 1952).

Effects of testosterone propionate on rat seminal vesicles. R. M. MELAMPY AND J. C. PORTER (introduced by E. A. HEWITT). *Iowa Agricultural Experiment Station, Iowa State College, Ames.*

Alkaline phosphatase activity of seminal vesicle tissue from normal male rats was 253 units and acid phosphatase was 107. A phosphatase unit is equal to mg. tyrosine equivalent to phenol liberated from disodium phenyl phosphate/gm. dry wt. tissue/hour. Three months after castration alkaline phosphatase decreased to 120 units and the acid to 11. Testosterone propionate (Perandren), 500 γ/day, was injected subcutaneously for 20 days. After 5 days of hormone treatment the

alkaline phosphatase increased to 861 units (618% increase over castrate level). Following this maximum, the activity decreased to a level of 511 units by the 20th day. Acid phosphatase increased 573% over the castrate during the first 5 days and 745% after 20 days. There was little, if any, secretion present in the castrate animal and the average dry weight of the gland was 48 mg. After 20 days treatment there was 1322 mg. of secretion and the dry weight of the gland was 525 mg. The composition of the secretion collected from normal animals was as follows: 25.9% dry matter; 12.6% total N; 0.44% N.P.N.; 1.6% ash; and 0.35% total reducing substances. Fructose was absent from both tissue and secretion. The Q_{O_2} of the castrate gland tissue was 3.5. Forty-eight hours after hormone treatment the Q_{O_2} rose to 6.2, decreasing to 4.2 by the fifth day and 3.4 by the twentieth day of the experiment.

Evaluation of drug protection against experimentally induced ventricular fibrillation in 499 dogs: Quinidine, Procaine, Pronestyl, Xylocaine, Trasentine, Pendiomide and Ciba #1194. GEORGE R. MENEELY, JAMES S. ANDERSON,* LESTER L. HIBBETT,* JAMES E. MCCARTY,* SAM E. STEPHENSON, JR.,* EDWIN R. YOUNG* AND CON O. T. BALL.* *Research Lab., Thayer Veterans Administration Hosp. and Dept. of Medicine, Vanderbilt Univ. School of Medicine, Nashville.*

We have previously reported the efficacy of quinidine in preventing ventricular fibrillation during 30-minute occlusion of the anterior descending coronary artery and upon release of the occlusion in Nembutal anesthetized dogs. Experiments with 6 additional drugs interspersed with controls on 499 dogs are here reported. The incidence of ventricular fibrillation among controls during the 30-minute occlusion was 26% and of the survivors, fibrillation occurred upon release of the artery in 71%. Quinidine provided highly significant ($P < 0.01$) protection both during occlusion and upon release; Procaine and Pronestyl significant ($P < 0.05$) protection during occlusion and Pronestyl suggestive ($P < 0.10$) protection on release. Xylocaine was ineffective. Trasentine, reported effected by other methods, did not protect. Of the 2 ganglionic blocking agents, Pendiomide (Ciba #9295) offered significant protection against ventricular fibrillation upon release of the occluded artery while tris diethylamino ethylamine hydrochloride (Ciba #1194) did not seem to afford any protection. (Supported in part by Ciba Pharmaceutical Products, Inc.)

Localization of cortisone in an inflamed area. VALY MENKIN. *Agnes Barr Chase Fndn. for Cancer Research, Temple Univ. School of Medicine, Philadelphia, Pa.*

Cortisone acetate (Merck) could be injected intravenously, very slowly, into rabbits in doses ranging from 25 to 165 mg., the latter being given in daily divided doses. An inflammation was induced, following the intravenous injection of cortisone, into the pleural cavity of rabbits with 0.2 cc. of 10% croton oil in olive oil. The exudate, containing both leukotaxine and presumably some exudin, was collected on the following day and also on successive days. Such exudative material was then injected into the skin of an otherwise normal rabbit, injected immediately before with 30 to 40 mg. of ACTH into the blood stream. An accompanying control rabbit was also utilized which had received 3 to 4 cc. of physiological saline intravenously. In essentially all cases, it was found that with an exudate derived from the pleural cavity of a rabbit injected with cortisone, tested in the skin of a rabbit injected in turn with ACTH, both the increased permeability of capillaries and the migration of leukocytes were suppressed, whereas in the control exudate from a rabbit injected with only saline intravenously, the trypan blue test appeared conspicuously, but not prominently when it was compared with the local accumulation of dye caused by exudative material taken from another control rabbit which had received no cortisone intravenously. The same suppressing effect was obtained from the exudate of a rabbit injected intravenously with cortisone when, however, the pleural inflammation had already been fully established. The latter would rule out in these experiments that cortisone inhibits the formation of leukotaxine, but rather indicates that cortisone by accumulating in the inflamed area exerts its suppressing effect on leukotaxine. ACTH injected intravenously into the experimental rabbit suppresses any exudin present in the tested exudates besides leukotaxine.

Cardiac glycogen in fishes during and following acute anoxia. A. W. MERRICK* AND M. M. ELLIS. *Dept. of Physiology and Pharmacology, Univ. of Missouri Med. School, Columbia.*

Changes in cardiac glycogen during anoxia were followed in goldfish, as they succumb in water of low oxygen content only after relatively long exposures. Three-inch goldfish were carried in 6-liter Erlenmeyer flasks, 4 fishes to a flask, at 19°C. The dissolved oxygen in water for anoxic series was reduced to 0.58 ppm by bubbling pure nitrogen gas through the water and holding the low oxygen water under this gas. Displacement of dissolved by O_2 by N_2 produced no significant changes in pH or salt balance in the well buffered tap water used. All control and revived fishes were carried in water of 7.4 ppm O_2. Cardiac glycogen was determined by the method of Somogyi, the average for the controls being 23.6 mg/gm. of cardiac tissue. Acute distress developed in fishes

abruptly transferred into the low oxygen water in approximately 5 hours. The end point for acute anoxia was set at 15 minutes after opercular movements ceased. In the anoxic series there was little or no change in cardiac glycogen before the symptoms of acute anoxia appeared; but with the onset of incoordination and irregular opercular movements cardiac glycogen fell rapidly to an average 2.6 mg/gm. Cardiac glycogen did not return to normal under 3 hours or more in anoxic fishes revived in fresh water, although after 6 hours cardiac glycogen again approached (19.6 mg/gm.) that of the controls. Thus during the first 3 to 6 hours following acute anoxia cardiac glycogen was about 11% of normal although swimming and respiratory movements appeared unaffected and revival seemed complete after 15 minutes.

Electrophysiologic phenomena evoked by electrical stimulation of caudate nucleus. FRED A. METTLER, CHRISTIAN A. HOVDE* AND HARRY GRUNDFEST. *Depts. of Neurology and Anatomy, College of Physicians and Surgeons, Columbia Univ., New York City.*

Inhibition of induced and spontaneous somatomotor activity upon repetitive electrical stimulation of the caudate nucleus has been described, as has inhibition and facilitation of autonomic activity. Inhibition of movement and suppression of electrical cortical activity have been associated with 'suppressor bands,' and firing of the caudate nucleus, following application of strychnine to the cortex. In the present study, it has not been found possible to activate caudate nucleus by electrical stimulation of cortex. Single shock electrical stimulation of caudate does, however, fire homolateral anterior and posterior sigmoid gyri of cat and, in monkey, discharges a region which has Brodmann's area 10 as its focal center. Conduction is rapid (response within 2 msec.). Cortical removal produces a reduction in size of the caudate nucleus and in the number and size of its cells. Single shocks delivered to the caudate are conducted in various directions. Stimulation of its ventricular surface results in slow, long sustained caudally directed conduction within the nucleus (traversing 5–6 mm. within 10–20 msec., response lasting over 30 msec. at caudal focus). Stimulation of ventral part of head of nucleus results in orally directed conduction having approximately the same characteristics. Characteristics of the response depend on loci of stimulation and recording. The caudate is not capable of responding in the above manner when stimulated repetitively (40–60 cycles). Results on hand indicate caudate consists of many multi-neuron chains and capable of prolonged activity.

Relation between dosage of thyroid hormone and metabolic increase in thyroidectomized rat used to determine the influence of iodinization on thyroid globulin. ARTHUR E. MEYER. *Fellows Pharmaceutical Labs., New York City.*

Thyroidectomized male rats have an oxygen consumption of 3.15 calories/kg/hour with a standard deviation of ±7.2%, except for the late spring months, when the metabolism is higher. Feeding graded doses of purified thyroid globulin elicits an increase following a logarithmic curve, if the responses are calculated in percentages of the pretreatment levels. Using the curve of the natural logarithm, a dosage unit is proposed that corresponds to the point at which the curve crosses the abscissa, below which the dose is at subthreshold levels. The unit of response is, then, that obtained at 2.7 dose units. Feeding 2.7 dose units for each 10 gm. rat on 2 consecutive days gave in these experiments a metabolic increase of 19% on the 4th or 5th day; 19%, therefore, is the response unit. One-half of purified thyroid globulin solution was treated with iodine in KI at 70° and precipitated isoelectrically, equally as the non-iodized portion. The natural thyroid globulin contained 0.82% I, the iodized 5.04%. Making allowance for the increase in weight by iodinization, equivalent doses were used for assay. The iodized product gave a curve closely coinciding with that of the non-iodized thyroid globulin. Iodinization did not add to the potency of the hormone. It is concluded that no free tyrosine groups were available in the molecule which on iodinization would enhance the potency of the molecule.

Effects of mercuric ion on sodium movement through the gills of gold fish. DALLAS K. MEYER. *Dept. of Physiology and Pharmacology, Univ. of Missouri, Columbia.*

Radioactive Na may be used to measure separately the rates of Na uptake through the gills of freshwater fishes and the simultaneous loss of Na through the gills that may occur in certain physiological states (*Federation Proc.* 10: 91, 1951). Measurements of Na uptake and Na loss were made over a 2-hour control period. Mercuric chloride was added to the aquarium water to produce the desired concentration and a second set of determinations taken 2 hours later. Mercuric ion has a profound effect on the movement of Na in both directions. The average rate of active Na uptake by the gills during the control period was 0.92 mg. Na/100 gm. of fish/hour. and the average rate of Na loss over the same period of time was 0.41 mg/100 gm. of fish. Mercuric ion in a concentration of 10^{-5} M completely inhibited the active uptake but increased the loss of Na to 4.02 mg/100 gm. of fish/hour. When the concentration of mercuric ion was reduced to 10^{-6} M the active uptake was depressed by 25% while the Na loss was increased by 43% over the control rates. An intraperitoneal injection of 5 mg. of 2,3-dithiopropanol protected a 40-gm. fish against both the inhibition of Na

uptake and the accelerated Na loss that followed exposure to 10^{-5} M mercuric ion.

No demonstrable glomerular-proximal tubule imbalance in chronic pyelonephritis. ALEXANDER J. MICHIE AND CATHARINE R. MICHIE (introduced by J. E. RHOADS). *Dept. of Physiology, New York Univ., College of Medicine, New York City and Harrison Dept. of Surgical Research, Schools of Medicine, Univ. of Pennsylvania, Philadelphia.*

Approximately the same time interval is required for the normal and nonhydronephrotic pyleonephritic kidney to reach equilibrium clearance of *p*-aminohippurate, phenolsulfonphthalein, and mannitol, thiosulfate, or inulin. With these substances equilibrium between plasma and urine occurs within 30 minutes in the normal kidney. A similar examination of 36 nonhydronephrotic pyelonephritic kidneys reveals that clearance equilibrium is attained in the same time. In 11 instances these measurements were obtained simultaneously in subjects having a hypertrophic normal and a pyelonephritic kidney. There was no significant difference in the time required for clearance equilibrium between the two kidneys. Also the ratio of one clearance to another in the diseased and nondiseased kidney in the same subject shows little variance provided T_{mPAH} is greater than 10 mg/minute. Finally the average of the various clearance ratios for 13 hypertrophic normal kidneys are very similar to the average of 36 nonhydronephrotic pyelonephritic kidneys whose T_{mPAH} is greater than 10 mg/minute. With this method we cannot demonstrate in chronic pyelonephritis a significant number of nephrons with only nonfunctioning glomeruli or only nonfunctioning proximal tubules.

Thermal radiation burns in rabbits. DAVID MINARD AND RODERICK E. JENSEN.* *Naval Med. Research Inst., Bethesda, Md.*

Shaved skin areas of albino rabbits weighing between 2.5 and 3.5 kg. have been exposed to a tungsten lamp source giving a maximum intensity of approximately 8.0 cal/cm²/sec. A threshold burn is produced in 0.3 second. An exposure of 0.6 second, produces a full thickness burn healing by scar formation. Exposures of 1.0 second and longer result in contraction of the skin during exposure followed later by sloughing and prolonged healing. Large area burns (approximately 7.5% of total surface area in 1 exposure or 15% in 2 exposures) were produced in rabbits anesthetized with ether. A transitory rise in hematocrit in the first 3 hours with a later fall below normal lasting many days is a consistent finding. The erythrocyte sedimentation rate becomes elevated in 24–48 hours after the burn and may return to normal well in advance of healing. A transitory fall in blood pressure with bradycardia seen during and immediately after exposure is considered to be reflex in origin. Secondary shock has not been observed. Normally hydrated animals exhibit no significant change in plasma volume. However, in animals maintained on a dry diet for 24 hours before burning, a reduction in plasma volume of 15–25% is seen 2 hours following the burn. The proportion of body surface which must be exposed to result fatally remains to be determined.

Cholesterol in the syrian golden hamster. M. C. MIROFF (introduced by P. A. WRIGHT). *Dept. of Zoology, Univ. of Michigan, Ann Arbor.*

Chemical determinations of cholesterol (Webb modification of the Sperry-Schoenheimer method) were carried out on the adrenals, ovaries, testes, brains, kidneys, livers, and sera of male and female hamsters. While the adrenals of all other animals which have been investigated to date, have had 70–80% esterified/total cholesterol, those of the hamster show no demonstrable amount of esterified cholesterol and only the usual amount of free cholesterol (0.368 mg/100 mg.). This free fraction, representing the cellular cholesterol, is lower than the amount found in the kidney (0.436 mg/100 mg.) or the testis (0.531 mg/100 mg.), but higher than in the liver (0.252 mg/100 mg.). The ovary and serum values averaged 73 and 68% esterified/total cholesterol, respectively.

Relation of trunk skin surface to digital skin surface temperatures of clothed men recumbent in dry-cold. GEORGE W. MOLNAR. *Army Med. Research Lab., Fort Knox, Ky.*

Two men in arctic uniform lay on mattresses for 2½–3 hours in air at −40°, with or without wind of 20 miles/hour. Rectal temperature showed either no change or a drop of 0.2°C. sometimes followed by a recovery of the same degree. During the first 20–75 minutes, trunk (chest, upper and lower back) skin surface temperatures dropped an average of 3.6° in wind and 2.8° in no wind, followed by rewarming averaging 1.9° in wind and 1.8° in no wind. The initial drop was partly due to contact with the initially cold mattress and subsequent rewarming was partly due to warming up of mattress (to 20°–26°C. at the end of the experiments). Rewarming was also partly due to the gradual rise in heat production during the 45–90 minute interval of exposure. Meanwhile the finger and toe surfaces cooled, with only occasional phases of rewarming, to reach 2.2° to −8.2°C. by the end of the experiment. The result was a not completely satisfactory regulation of bodily temperatures. While the trunk was kept comfortable, the extremities became painful and were in danger of freezing. Tripling instead of doubling the heat production could probably have

maintained the digits comfortable. Sacrifice of some trunk heat to the digits would not have been unbearable or deleterious.

Localized lesions in the bulboreticular inhibitory formation produced by ultrasound. W. H. Mosberg, Jr., D. Tucker and W. J. Fry. (introduced by W. S. McCulloch). *Manteno State Hosp., Manteno, Ill. and Dept. of Electrical Engineering, Univ. of Illinois, Urbana.*

The effect of exposing nerve tissue to high intensity ultrasound for short periods has been described (*Science*, Dec. 1951). In these experiments a single, 1 mm. diameter focused ultrasonic beam was directed at the area of the bulboreticular inhibitory formation. The crystal generating head was arranged so that the beam was directed at coordinates in a Horsley Clarke stereotactic instrument. The ultrasonic frequency was 1.0 megacycle. The duration of irradiation was 1.0 second. The acoustic pressure amplitude and duration were chosen to destroy only nerve cell bodies with a preferential destruction of large cells. Up to 20 adjacent areas were irradiated to cover the whole of the inhibitor formation. Twenty-two cats were irradiated; recovery from operation and anesthesia was complete in 24–48 hours. All cats showed a permanent deficit with a resting posture of extended fore limbs and slightly flexed hind limbs. On walking, the fore limbs showed a goose step and the hind limbs a wide base with circumduction. The fore limbs showed an exaggerated grasp reflex. Histology of the ultrasonic lesions and tract degeneration was examined. The lesion was found well localized and confined to cell bodies except in 2 animals where vascular damage occurred. The nature of the lesion and the course and termination of the bulboreticular fibers will be reported.

Residual air volumes in the supine and standing positions. Hurley L. Motley. *Cardio-Respiratory Lab., Barton Memorial Div., Jefferson Hosp., and Dept. of Medicine, Jefferson Med. College, Philadelphia, Pa.*

The residual air volume was measured in the supine and standing positions with the oxygen open circuit method in 40 cases with pulmonary fibrosis and emphysema. A mean average decrease of 0.21% in the standing residual air percentage of total lung volume (TLV) with a S.D. of 5.2% was observed. In *group 1* (26 cases with an average supine residual air 49.9% of TLV and all above 35%) there was an average decrease of 2.5% in the standing position (14 were decreased and 12 were increased). In *group 2* (14 cases with an average supine residual air 29.9% of TLV and all below 35%) there was an average increase of 4.1% in the standing position (all cases were increased). In *group 1* the average residual air volume was increased from 2602 cc. supine to 2704 cc. standing, while in *group 2* the increase was from 1784 cc. supine to 2228 cc. standing. The average standing vital capacity was increased 331 cc. in *group 1* and 122 cc. in *group 2*. The average expiratory reserve was increased about 500 cc. standing. The average mid capacity (residual air plus expiratory reserve) percentage TLV was increased 4.37% in *group 1* and 11.07% in *group 2* in the standing positions, and the individual variations by this expression were much greater as compared to designating the residual air as percentage of total lung volume. Changes in the residual air percentage of TLV in the standing position in man are of small magnitude as compared to supine.

Activity of single neurons of the somatic sensory nuclear complex of the thalamus. Vernon B. Mountcastle and Jerzy E. Rose. *Johns Hopkins Univ. School of Medicine, Baltimore, Md.*

The activity of single neurons of the ventrobasal thalamic complex has been recorded with 2–4μ glass micropipettes filled with metal. Cutaneous stimulation (air jet, brush, electrical) evokes at appropriate thalamic loci potential changes characterized by a train of 2–4 spike action potentials of 0.4–0.6 msce. duration. By all available criteria these spikes are discharges of the same single unit. Recovery studies show that *1*) at short intervals two stimuli evoke a longer train of spikes than that evoked by either alone, and *2*) the relative refractoriness seen at longer intervals may be defined in terms of reduction in the number of discharges evoked by the second stimulus. Frequency studies divide the unit population into two groups. Those of the first follow stimuli to 30–40/second, and equilibrate at that level for higher rates. The second group of units follows stimulation up to a certain frequency. With further increase of stimulus rate they cease to discharge after an initial burst. In terms of modality the units fall into three groups: those activated by hair movement, by cutaneous pressure, or by mechanical stimulation of the periosteum and movement of joints (but not by stimulation of skin or stretch of muscle). Different receptors located peripherally in close proximity may activate different thalamic units which lie no further apart than the order of a cell diameter. Among the many hundreds of units observed none was encountered which could be activated by stimulation of the ipsilateral trunk or extremities.

Electrolyte metabolism of rabbit kidney cortex slices. Gilbert H. Mudge. *Dept. of Medicine, Columbia Univ. College of Physicians and Surgeons, New York City.*

Previous *in vitro* studies with kidney slices have demonstrated that K accumulation and reciprocal

Na extrusion are dependent upon aerobic oxidation and the availability of phosphate bond energy. In continuation of these studies the dynamic aspects of transport have been examined with radioactive isotopes under a variety of metabolic conditions. Under optimal *aerobic* conditions, K exchanges completely within 60 minutes at 25°C. *Anaerobically* the initial rate of K exchange is less rapid, and complete exchange does not occur; approximately 40% of tissue K does not exchange after as long as 4 hours' incubation. The results indicate that under *anaerobic* conditions tissue K exists in 3 functional states: *1*) exchangeable extracellular K, *2*) exchangeable intracellular K, and *3*) nonexchangeable K. Since complete exchange occurs *aerobically* and not anaerobically it follows that the functional state of a fraction of tissue K is conditioned by metabolic reactions. Supporting indirect evidence indicates the nonexchangeable fraction is chemically bound K. Further studies are in progress on Na exchange and the effects of metabolic inhibitors.

Anemia of renal insufficiency (uremia) as observed in dogs following bilateral nephrectomy and maintenance by peritoneal irrigation: Evidence for a hemolytic mechanism. E. E. Muirhead, J. A. Stirman, F. Jones and W. Lesch (introduced by Carl A. Mayer). *Southwestern Med. College, Dallas, Tex.*

Nephrectomies were performed, 1 week apart, on 17 dogs. Following the second nephrectomy the peritoneal cavity was irrigated with a crystalloid solution having a composition similar to protein-free plasma and containing terramycin. Azotemia was partly controlled and the survival was extended. Observations were conducted for 5–22 days. The hematocrit reading decreased. The red blood cell volume, as measured by the T1824-hematocrit method, decreased strikingly. The anemia was associated with an elevation of serum bilirubin (both 1-minute and 45-minute values) and an elevation of serum iron and serum iron binding capacity (siderophilin). Eight animals studied revealed an increase in the daily excretion of urobilinogen in the stool. The spleen, lymph nodes and liver revealed hemosiderosis. Erythrophagocytosis was evident in the lymph nodes. The total quantity of iron in the peritoneal fluid removed and in the stools was negligible. No excessive hemorrhages were noted at autopsy and the amount of blood removed as samples was meager. It is concluded that a considerable anemia develops after bilateral nephrectomy of the dog and that the rapidity of development plus the other observations reported indicate the operation of a hemolytic mechanism as its main cause. These findings are similar to prior observations made on the rabbit.

Effect of adrenaline on the concentration of the energy-rich phosphates and of the glycogen of the dog heart. A. G. Mulder, Akira Omachi,* Blanche Tigerman* and Michael Ruane.* *Dept. of Physiology, Stritch School of Medicine, and Graduate School, Loyola Univ., Chicago, Ill.*

The experiments of others on intact animals have shown either an increase or a decrease in the cardiac glycogen resulting from the infusion of adrenaline. (In isolated hearts (Bogue, Evans and Gregory) the glycogen has been found to be very low as a result of adrenaline infusion.) We have determined the effect of adrenaline on the cardiac glycogen given to the intact dog and extended these observations to include its effect on the energy-rich phosphates, lactic acid, sodium, and potassium concentrations in the myocardium. Adrenaline was injected in various doses but usually about 1 mg/kg. dog/hour. The infusion lasted about 2 hours. The blood pressure was elevated and was usually maintained over 200 mg. Hg for most of the 2-hour period. The work of the heart was thus greatly increased. Analysis of the heart showed marked reduction in glycogen concentration, marked reduction in the concentration of adenosine polyphosphate with a tendency for an increase in the concentration of phospho-creatine.

Polarographic differentiation of various plasma albumins. Otto H. Müller, Lester H. Gershenfeld* and Mary Jane Elwood.* *Dept. of Physiology, State Univ. of New York Med. Center, Syracuse.*

Plasma albumins of man and several animals can be differentiated polarographically as follows. To 1.0 ml. of the albumin solution (0.5–5% protein in 0.9% sodium chloride) add 0.25 ml. 1.0 N potassium hydroxide, mix and note the time. For the next hour, remove, at intervals of 5 or 10 minutes, aliquots of 0.05 ml. from this mixture, add them to 10 ml. of divalent cobalt test solution (Müller and Davis, *Arch. Biochem.* 15: 39, 1947), and polarograph immediately. The polarographic waves rise very rapidly during the first 5 minutes of digestion and fall subsequently at a slower rate for about 60 minutes. After that time they decrease at a new, still slower rate. If the *logarithm* of the polarographic current density is plotted *vs.* time of digestion, a straight line can be drawn through the points lying between 10 and 60 minutes. From the slope of this line a characteristic velocity constant or half life can be calculated for each protein analyzed. We found such half lives to be *a*) consistent for a given albumin, *b*) independent of the protein concentration, and *c*) varying sufficiently between available human, bovine and porcine albumins to make differentiation possible. The samples used represent pooled

or 'normal' specimen and hence may have a slightly different half life than *fraction V* of any individual plasma. Such differences could reflect abnormalities in the albumin which may be related to disease. Results obtained in several individual cases will be discussed from this aspect.

Nerve metabolism as studied with C-14 labelled compounds. L. J. MULLINS (introduced by W. R. AMBERSON). *Purdue Univ., Lafayette, Ind.*

An apparatus is described which permits the measurement of CO_2 evolved by the breakdown of radio carbon labelled compounds by frog sciatic nerve. Comparative measurements in resting and stimulated nerve indicate that glucose and acetate-C-14 do not produce as much CO_2 as would be calculated from O_2 uptake measurements.

Temperature effects on tissue chemistry of the Arctic sculpin. XAVIER J. MUSACCHIA,* MARGUERITE CLARK* AND CHARLES G. WILBER. *Biological Labs., St. Louis Univ., St. Louis, Mo.*

As part of a research program dealing with metabolism of Arctic animals, lipids and glycogen of liver and skeletal muscle from the fish, *Myoxocephalus quadricornis*, were investigated. Studies were made on these constituents after subjecting sculpins to temperatures greater than that of their natural habitat (2°–3°C.). Before interpreting changes of tissue constituents, normal ranges of values were established. Standard biochemical quantitative methods were used to estimate the various lipids. The average values (in percentage wet weight) obtained for lipids of the liver tissue from 15 control specimens are: phospholipid, 4.02%; total cholesterol, 0.37%; cholesterol esters, 0.32%; total fatty acids, 12.80%. The average values for skeletal muscle are: phospholipid, 1.85%; total cholesterol 0.07%; cholesterol esters, 0.03%; total fatty acids, 1.35%. There was a marked increase in total cholesterol and cholesterol esters, 1.11% and 0.93% respectively, of the liver from specimens maintained at a water temperature of 20°C. Histochemical (Bauer-Feulgen) studies of liver showed that at 10°C. the majority of specimens (11 out of 12) had increased concentrations of glycogen. At 20°C. 5 out of 7 specimens had increased concentrations of liver glyocgen. The chief alteration in skeletal muscle occurred at 20°C. There was a pronounced decrease in phospholipid. This fact was correlated with diminished swimming movements of the fish. There did not appear to be any significant changes in muscle glycogen of fish at elevated temperatures.

Effects of pancreatic hyperglycemic factor on hepatic carbohydrate metabolism of man. J. D. MYERS, ROBERT F. KIBLER,* AND W. JAPE TAYLOR.* *Dept. of Medicine, Duke Univ., Durham, N. C.*

Pancreatic hyperglycemic factor (HGF) has been shown to produce hyperglycemia in man and animals by means, in animals at least, of promoting glycogen breakdown in the liver. In the present study, HGF (Lilly) 5 mg. as a single intravenous injection in 6 fasting control human subjects caused a mean maximum rise in arterial blood glucose of 21 ± 1.7 (S.E.) mg. %, the peak occurring at 10 minutes after HGF. Concomitantly, glucose concentration in hepatic venous blood (obtained by catheterization) increased by 36 ± 1.2 mg. % with the peak between 2½ and 5 minutes. The hepatic venous-arterial blood glucose difference increased from a mean of 6.3 ± 0.6 mg. % fasting to 23.4 ± 1.2 mg. % between 2½ and 5 minutes after HGF. Since HGF had no effect on hepatic blood flow, there resulted an over 3-fold rise in splanchnic glucose production. This was not associated with any alteration in splanchnic oxygen consumption. In 13 studies on 10 patients with extensive diffuse parenchymatous liver disease (Laennec's cirrhosis, acute and chronic hepatitis, and biliary cirrhosis), where a reduced hepatic glycogen content might be expected, the response to HGF was significantly subnormal in the arterial blood, where the mean maximum rise in glucose, at 10 minutes, was only 5 ± 1.1 mg. %. Four individuals with liver disease had hepatic venous catheterization and again subnormal outputs were found. In summary, HGF promotes a rapid and substantial rise in blood glucose in man by augmenting splanchnic glucose production. Diffuse liver disease is usually associated with a strikingly subnormal hyperglycemic response, suggesting that HGF may provide a quite simple test for hepatic glycogen content.

Studies on the physiological status of bone carbonate. AKIRA NAKAO AND DONALD L. BUCHANAN (introduced by LESTER R. DRAGSTEDT). *Div. of Biological and Med. Research, Argonne Natl. Lab., Chicago, Ill.*

During isotopic turnover studies it was noticed that the specific activity (*s.a.*) of bone carbonate diminished during manipulations preceding analyses. To study this phenomenon rats were sacrificed after an exposure to $C^{14}O_2$ in air for 2 weeks, during which time only 35% of the bone carbonate had exchanged with the labeled CO_2-bicarbonate pool. In bones of these animals the carbonate *s.a.* diminished to the indicated percentages (of untreated controls) in the following operations: extraction with ethylenediamine (1 hr.), 54%; boiling in 3% KOH in glycol (90 min.), 66%; boiling in distilled water (1 hr.), 91%; and evacuation below 1 micron (3 days), 98%. The CO_2 evolved during the 3-day evacuation had the same *s.a.* as the CO_2-bicarbonate pool. Bones from animals exposed for different periods were ignited at 500° for 24 hours.

The fractional diminution in s.a. with this treatment was proportional to the fractional replacement of carbonate by isotope during the exposure. These findings and others indicate that bone carbonate recently exchanged *in vivo* is more susceptible than older carbonate to removal by chemical and physical agents. To determine whether this time dependent behavior results from diffusion through the bone matrix or from heterogeneity of carbonate within the bone salt ground samples of this bone were fractionally acidified. The CO_2 evolved first had a high *s.a.* which diminished with each successively liberated fraction. This finding was independent of particle size down to 1 micron. We conclude that the carbonate heterogeneity lies in the bone salt.

Electrocardiograms of the opossum at low temperatures. ROLAND M. NARDONE AND CHARLES G. WILBER (introduced by R. H. K. FOSTER). *Biological Lab., St. Louis Univ., St. Louis, Mo.*

Opossums were trapped, anesthetized lightly with sodium nembutal, and exposed to decreasing ambient temperatures. Electrocardiograms were recorded every 5 minutes via leads I, II, III, AVR, AVL, and AVF. It was observed that there is not a straight line decrease in the heart rate with a decrease in colonic temperature; the heart rate decreases in a step-wise manner from 240/minute to 0. No heart beats were detectable with the electrocardiogram after the colonic temperature reached 10°C. Most animals perished at temperatures 3°–5°C. higher than this minimal temperature. The factors involved in the decrease of the heart rate were an increase in the S-T segment (a prolonged depolarized state) from 0.06–0.20 sec. and an increase in the P-R segment (A-V node delay) from 0.02–0.08 sec. Specimens that were damaged beyond recovery showed an increase in the length of the QRS complex and T wave (indicating slower myocardial conduction). The intensity of the spike increased simultaneously with a decrease in colonic temperature until a critical temperature was reached, after which the heart beat became erratic and feeble. The critical temperature was about 15° ± 4°C. It varied with each animal. Some recordings revealed arrhythmias, auricular flutters and fibrillation. There was no consistency in these disorders. Lead I T wave became diphasic (− + type) in one specimen after the colonic temperature reached 13°C. The same specimen displayed right axis deviation at the same temperature. One specimen had an inverted T wave after the colonic temperature decreased to 19°C. Recordings from all leads except lead I showed an increase in the intensity of the S wave in all specimens.

Organization of the central respiratory mechanism in cats. S. H. NGAI,* M. J. FRUMIN* AND S. C. WANG. *Depts. of Anesthesiology and Physiology, College of Physicians and Surgeons, Columbia Univ., New York City.*

The pons and medulla were transected serially in 85 *vagotomized*, decerebrate cats to reascertain the locations and functions of the respiratory center complex. Rhythmical breathing persisted when the transection extended to the rostral pontile border dorsally and about 2 mm. within the pontile protuberance ventrally. Transections between this plane and the mid-portion of trapezoid body produced apneustic breathing interrupted by expiratory spasm and gasps. Within these limits if the transection is high, prolonged apneusis (6 minutes) predominates; if the level is low, expiratory apnea alternating with transient apneusis prevails. These findings suggest that an additional respiratory regulating mechanism resides in the caudal two thirds of the pons. Transection caudal to the mid-portion of the trapezoid body resulted in gasping or Biot's breathing, adequate for survival for several hours. The lowest level of transection resulting in periodic respiration is the upper border of the calamus scriptorius. The medullary respiratory centers have an autonomous rhythm when released from the influence of the pneumotaxic and other pontile centers and afferent vagal impulses. The medullary inspiratory center is not continuously, tonically active as Pitts *et al.* suggested. Electrical stimulation experiments using a stereotaxic instrument revealed that the pneumotaxic center is located in the tegmentum of the upper few millimeters of the pons. Inspiratory spasm was observed on stimulating the lateral reticular formation of the mid-pons and expiratory apnea at the corresponding area of the caudal pons. These loci are probably responsible for the apneustic type of breathing observed in the above transection experiments.

Effect of adrenal cortical atrophy on the course of alloxan diabetes. JOHN NICHOLS AND H. L. SHEEHAN.* *Dept. of Pathology, Univ. of Liverpool, Liverpool, England.*

The adrenal cortical atrophy which results from administration of DDD (2,2-bis(parachlorophenyl)-1,1-dichloroethane) to the dog involves the loss of the zona reticularis and most of the zona fasciculata. The histological appearance is almost identical with that of human patients with hypopituitarism. It has been previously reported (*J. Lab. & Clin. Med.* 37: 229, 1951) that such dogs are relatively insulin sensitive. The response of such dogs to alloxan would, therefore, be of interest. Five dogs weighing from 5–10 kg. whose cortices had been made atrophic by 5 weeks of DDD administration were given alloxan and blood sugar determinations made at 2-hour intervals

for the next 36 hours. The initial hyperglycemia was much less and the subsequent transient hypoglycemia occurred earlier and was more profound in the DDD-treated dogs than in dogs with intact adrenals. Blood sugar determinations were made daily for about a week after recovery from the hypoglycemic phase. During this time the blood sugar in the DDD-treated dogs ranged about 120–150 mg.% while in dogs with intact cortices it ranged about 300–350 mg.%. After this time the dogs with atrophic cortices were given a potassium tolerance test consisting of 2 oral doses of 5 gm. potassium chloride with a 6-hour interval. No signs suggestive of defects in electrolyte control were observed. This suggests that in the dog the outer zones of the adrenal cortex are concerned with electrolyte balance while the inner zones are concerned with carbohydrate metabolism.

Spectrophotometric determination of T-1824 in whole hemolyzed blood. JOHN W. NICHOLSON, III AND GABRIEL G. NAHAS (introduced by NORMAN M. KEITH). *Mayo Fndn., Univ. of Minnesota, and Mayo Clinic, Rochester.*

A chamber, designed for the Beckman spectrophotometer, and a technic for laking samples of oxygenated blood with saponin have permitted measurement of T-1824 in whole hemolyzed blood, thus avoiding the necessity for hematocrit determinations in dye dilution procedures. Optical densities of dyed samples were determined at 620 mμ against undyed samples of blood from the same source. These readings were converted to concentrations by use of the straight calibration line found to span the range encountered in clinical blood-volume studies (3–25 mg. dye/liter). Standard deviation of points from this line was 2%. Thirty *in vitro* comparisons between blood volumes determined volumetrically and by dilution of T-1824 in hemolyzed blood gave a mean value of 102.5% of the volumetric measurement, standard deviation of 5.4. With use of this technic, 16 simultaneous *in vitro* determinations of blood volume were made by the plasma volume-hematocrit method, the hemolyzed blood method, and direct volumetric measurement. The mean value for blood volume obtained by the plasma method was 101% of the volumetric measurement, standard deviation 7%. The mean difference between values for blood volume calculated from the plasma method and the hemolyzed blood method was 3%, standard deviation of the differences being 8.9%. Studies made during cardiac catheterization, with use of the hemolyzed blood method, showed no systematic difference in dye concentrations in arterial and peripheral venous blood, but less dye was noted in mixed venous blood when the samples were withdrawn simultaneously 5 minutes after intravenous injection of the dye.

Effect of pressor agents on renal water and electrolyte excretion in man. J. F. NICKEL,* C. McC. SMYTHE* AND S. E. BRADLEY. *Dept. of Medicine, Columbia Univ. College of Physicians and Surgeons, and the Presbyterian Hosp., New York City.*

The intravenous infusion (15 μg/min.) of *l*-norepinephrine or *l*-epinephrine causes renal vasoconstriction and decreased renal blood flow without change in glomerular filtration rate in human subjects. In addition, there is augmented tubular reabsorption of sodium and potassium and decreased tubular reabsorption of water. The intravenous infusion of a smaller amount (2 μg/min.) of *l*-epinephrine in 2 normal subjects produced the same changes without alteration in blood pressure or pulse rate. The change in electrolyte excretion is apparently not mediated through neural pathways or through adrenal cortical activation. In 7 normal subjects high spinal anesthesia (2% nupercaine) did not alter the action of *l*-norepinephrine. Two patients with adrenal cortical insufficiency responded like normal subjects to *l*-norepinephrine. Diminished formation of antidiuretic hormone may account for the decreased tubular water reabsorption. In 1 patient with diabetes insipidus *l*-norepinephrine depressed urinary electrolyte excretion, but urine volume fell. In 5 normal subjects administration of *l*-norepinephrine during antidiuresis induced by pitressin infusion (1 mμ/kg/hr.) resulted in diminished electrolyte excretion without change in urine flow. Angiotonin and s-methyl isothiourea also appear to depress sodium and potassium excretion in man. Epinedrine (3 mg/min.) in 4 normal subjects also induced renal vasoconstriction which was not sufficient, however, to affect renal blood flow. Like the medullary hormones, it diminished potassium excretion and increased the urine flow. Sodium excretion always increased.

Pressure-volume measurements upon the lungs and thorax. ROBERT G. NIMS,* STELLA Y. BOTELHO,* OVE NISELL* AND J. H. COMROE, JR. *Dept. of Physiology and Pharmacology, Graduate School of Medicine, Univ. of Pennsylvania, Philadelphia.*

Pressure-volume studies on the lung-thorax system were carried out on 20 patients deeply anesthetized prior to surgical procedures. A cuffed endotracheal tube was inserted and temporary apnea was produced by hyperventilating and/or curarizing the patients. The lungs were then inflated to different degrees and the tube occluded. The static pressure was measured at each level following which the volume increments were measured by allowing the lungs to empty to the resting expiratory level into a compensated 6-liter spirometer which recorded upon a rapidly moving kymo-

graph. ΔP was plotted against $\Delta V/VC$ or $\Delta V/$ estimated *FRC*. In several subjects, relaxation pressure curves obtained in the conscious 'relaxed' subject were compared with the P-V curves obtained in the same individual after muscular relaxation was induced by deep anesthesia and curarization. P-V data and expiration curves were also obtained on 11 dogs and 8 cats, under varying combinations of the following conditions: closed thorax, open thorax, light anesthesia, deep anesthesia, curarization, congestion of the lungs, exsanguination of the lungs, cardiac arrest for periods of 2 hours, bronchoconstriction produced by aerosols, chemical irritation of the respiratory tract or inhalation of hot air. In order to relate ΔV to *FRC* under conditions of rapidly changing *FRC*, gas dilution and pneumatometric methods for rapid estimation of *FRC* were evaluated in anesthetized animals. Determination of *FRC* permits estimation of changes in tissue elasticity as well as measurements of elastance of the lung or of the lung-thorax system.

Effects of cold exposure on brain activity. WERNER K. NOELL, STANLEY A. BRILLER* AND WILLIAM B. BRENDEL.* *USAF School of Aviation Medicine, Randolph Field, Tex.*

While recording the electrical activity of various brain regions, the electrocardiogram, respiration, arterial blood pressure and body temperature, hypothermia was produced in unnarcotized rabbits by immersion in cold water (5°–12°C.). Responses to various sensory, electrical and chemical stimuli were tested. In contrast to the depressive effects associated with hypothermia, certain distinct 'stimulatory' phenomena were strikingly evident. At body temperatures below 30°C. the threshold for the induction of cortical seizures was found to be markedly lowered. In chilled animals, paroxysmal activity of the striate area spreading over the entire cortex and of long persistence was evoked by electrical lateral geniculate stimulation of a strength and frequency ineffective before and after cooling. High spinal transection before immersion failed to alter this abnormal response pattern. Much the same as electrical stimulation, intravenous metrazol, caffeine and coramine, in doses subconvulsive during the euthermic state, produced cortical seizures. These chemical stimulants also revealed abnormal responsiveness of the cardio-vascular and respiratory systems during hypothermia. In low doses they invariably produced respiratory arrest which developed simultaneously with motor convulsions. The arrest, occasionally preceded by a transient increase in frequency and decrease in volume of respiration, extended beyond the period of motor convulsions and tended to produce fatal anoxia. The apnea was independent of variable abnormal cardio-vascular reactions such as bradycardia and hypotension. Abolition of rhythmic respiration was also the most frequent result of electrical stimulation of pontine regions during cold. Characteristically this apnea was of long duration and exceeded the period of exaggerated muscular spasms.

Effect of roentgen irradiation upon growth and peripheral blood cell levels of the albino rat. THOMAS R. NOONAN AND ANN M. NOONAN (introduced by JOE W. HOWLAND). *Dept. of Radiation Biology, Univ. of Rochester, School of Medicine and Dentistry, Rochester, N. Y.*

Three-week-old, albino rats, both male and female, were exposed daily or weekly to whole-body roentgen irradiation. The effect of these procedures upon growth (as measured by body weight) and upon the levels of hemoglobin, neutrophils and lymphocytes in the peripheral blood was studied. Exposure to 100 roentgens per week, either as a single dose or in 5 equal daily doses, for 4 weeks resulted in a significant decrease in growth and in absolute lymphocyte counts in both males and females. This procedure did not result in any significant change in hemoglobin levels or in absolute neutrophil counts in a period of 10 weeks of exposure. Exposure to 10 roentgens per day 5 times weekly produced a significant decrease of growth of male but not of female rats during the 10-week period. The changes in peripheral blood cell levels of normal albino rats between the 3rd and 13th week of life include an increase in hemoglobin concentration, an increase in absolute lymphocyte counts and little change in absolute neutrophil counts. (This is based on work performed under contract with the U S. Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, N. Y.)

Method for determination of lung compartments and bellows function in humans. ARTHUR H. NORRIS,* MILTON LANDOWNE AND NATHAN W. SHOCK. *Natl. Heart Inst., Natl. Insts. of Health, Bethesda, and Sect. on Gerontology, Baltimore City Hosp., Baltimore, Md.*

Functional residual capacities in recumbent resting subjects were determined by a wash-out method with the following advantages: *1)* Minimal cooperation of the subject is required. *2)* Concurrent estimates of respiratory exchange may be made. *3)* Method is insensitive to changes in respiratory pattern. Nitrogen was washed from the lungs by inspiration of a mixture of 80% helium and 20% oxygen. Helium was then washed out with atmospheric air. Ten-minute estimates of O_2 consumption and CO_2 production were made before, during and after wash-outs. The O_2 and CO_2 concentrations were determined by Haldane

analysis. Helium concentrations in expired air were recorded continuously with a katharometer. Spirograms were recorded with subjects recumbent. Voluntary ventilatory capacities were recorded with subjects standing. All measures were made on each of 2 days from 2–77 days apart (average, 15 days) in a preliminary sample of 12 laboratory workers (aged 25–60 years) and 15 ambulatory patients (aged 49–86 years) from the Infirmary Division, Baltimore City Hospitals. The standard error of estimate of *day 2* from *day 1* was ±0.26 l. for functional residual capacity (mn., 1.58 l.); ±0.33 l. for total lung capacity (mn., 3.64 l.); ±0.28 l. for residual volume (mn., 0.88 l.); ±14.07 l. for voluntary ventilatory capacity (mn., 81.41 l.). The 0.9 l. dead space of the apparatus was determined by helium wash-out with an accuracy of 1%.

Relation of pyridoxine deficiency and hypertension in the rat. NORMAN S. OLSEN AND WILLIAM E. MARTINDALE.* *Dept. of Biochemistry, Vanderbilt Univ. and Research Lab., Thayer VA Hosp., Nashville, Tenn.*

The present investigation is concerned with the possible role of pyridoxine deficiency in the production of hypertension. Male albino weaned rats were used and initially divided into three groups in regard to diet; the first received Purina checkers, the second, a purified complete diet, and the third, a diet deficient in pyridoxine with a supplement of the antimetabolite desoxypyridoxine. Growth curves, food intake and blood pressures were studied, and at termination the relative weights of adrenal, kidney, heart and liver were determined. It was found that after 4 months the weights of the three groups had plateaued and that while the blood pressures of the animals on the adequate diets were within narrow normal limits (118–120 mm. Hg systolic pressure), the blood pressures of the animals on the pyridoxine deficient diets were scattered over a wide range (109–158 mm.) showing a highly significant mean increase of 15 mm. Hg. A subgroup of each of the initial groups was made at the time the weights had become constant. The first, on Purina checkers, received desoxypyridoxine daily by stomach tube. Although these animals exhibited consistent increases in blood pressure the mean increase was not significant. The second subgroup was placed on essentially the original deficient diet and showed highly significant increases in blood pressure as well as clinical symptoms. To the third subgroup further desoxypyridoxine was given in the diet; no further increases in blood pressure were noted in these already deficient animals. A group of the hypertensive deficient animals was placed on an adequate diet with a supplement of pyridoxal. A rapid increase in weight together with a highly significant fall (mean fall 21.3 ± 3.2 mm. Hg) in blood pressure to normal levels was observed.

Induction period of photosynthesis in *Chlorella*. RODNEY A. OLSON AND FREDERICK S. BRACKETT (introduced by PAUL D. ALTLAND). *Natl. Insts. of Health, U. S. Public Health Service, Bethesda, Md.*

The time course of photosynthesis and respiration in *Chlorella* was observed by recording, at 10-second intervals, the oxygen tension in a suspension of cells during alternate dark and light periods with a method (*J. Gen. Physiol.* 32: 681, 1949) involving time selection and alternating potential on a static platinum electrode. This method provides high sensitivity and time resolution not afforded by other techniques. The light was directed through opposite windows of the cuvette containing the cells such that all cells received nearly equal energy in suspensions of up to 30% absorption (*Biol. Bull.* 97: 271, 1949). Under normal conditions the rate curve of oxygen evolution in *Chlorella* shows, on illumination, first a sharp and almost linear rise which quickly levels off to a steady rate. For suspensions dark-adapted 12 hours or more this process may require several minutes, whereas in light-adapted suspensions it may occur in 10–20 seconds. The development of adaptation is evident in successive light and dark alternations of 1–6 minutes. In suspensions dark adapted under anaerobic conditions a delay of perceptible oxygen evolution up to 2 minutes may occur, depending on the length of dark adaptation. Dark adaptation of 1–3 hours shows maximum delay while prolonged darkness (18–24 hours) shows no delay. Once oxygen evolution begins, the approach to the final steady rate is similar to that occurring under aerobic conditions. Representative data and curves will be presented and discussed with reference to recent claims regarding high initial quantum efficiency.

Effect of lithium on acidification of the urine. JACK ORLOFF AND THOMAS J. KENNEDY, JR. (introduced by HEINZ SPECHT). *Section on Kidney and Electrolyte Metabolism, Natl. Heart Inst., Natl. Insts. of Health, Public Health Service, Federal Security Agency, Bethesda, Md.*

Competition between potassium and hydrogen ions for secretion by the renal tubules has been proposed to explain certain relationships between potassium metabolism and urine acidification, particularly the effect of inhibitors of carbonic anhydrase. Foulks, Mudge, and Gilman (*Federation Proc.* 9: 41, 1950) have shown that lithium enhances the secretion of potassium, but does not inhibit red cell carbonic anhydrase (Mudge, personal communication). With the view that an

agent which increases the secretion of potassium should exert an opposing effect on the acidifying mechanism, lithium chloride in an acid phosphate buffer was administered to acidotic dogs by the intravenous route. Coincident with the rise in the urinary excretion of potassium, the secretion of hydrogen ion diminished as evidenced by a rise in pH of the urine, an increase in bicarbonate excretion, and a fall in titratable acidity. These changes occurred despite a fall in plasma bicarbonate in some of the dogs. The results tend to substantiate the thesis concerning the competition between H^+ and K^+ ions for secretion and support the view that such effects need not be mediated by the carbonic anhydrase system.

Kinetics of muscle adenosine triphosphatase. LUDOVIC OUELLET AND KEITH J. LAIDLER (introduced by E. HARDENBERGH). *Div. of Physical Biochemistry, Naval Med. Research Inst., Bethesda, Md.*

The kinetics of the dephosphorylation of ATP by 'myosin' (Weber-Edsall type, in .6 M KCl) follow the law, $-d[ATP]/dt = k_2K[myosin][ATP]/(1 + K[ATP])$, at pH 7.0 and 10^{-3} M Ca^{++} The apparent free energies, heats and entropies associated with K and K_2 are, respectively, $\Delta F = -6.5$ kcal mole^{-1}, $\Delta H = 8$ kcal mole^{-1}, $\Delta S = 49$ cal mole^{-1} deg^{-1}, $\Delta F_2^{\neq} = 14.8$ kcal mole^{-1}, $\Delta H_2^{\neq} = 12.4$ kcal mole^{-1}, and $\Delta S_2^{\neq} = -8.0$ cal mole^{-1} deg^{-1}, if a monovalent enzyme of molecular weight 2.0×10^7 is assumed, and k_2 (rate constant for products formation from myosin-ATP complex) has the Eyring form. There is some evidence that K is actually the equilibrium constant for the complex formation. The measured ΔS suggests that this formation involves extensive charge neutralization, and lends some support to the idea that ATP neutralizes a positive charge on the myosin, allowing it to coil to a more probable configuration. There are also presented certain theoretical considerations of pH and Ca^{++} effects which allow a more rigorous interpretation of these data and are of interest to kinetic analyses of other enzyme systems. Extrapolation of *in vitro* dephosphorylation rate data to the *in vivo* system is tenuous because of important unknown differences between the two systems; an illustration of these differences is the great thermal instability of the former and the stability of the latter.

Interrelation of circulatory mixing and transcapillary exchange using T-1824, Na^{24} and K^{42}. RICHARD R. OVERMAN AND A. KENNON DAVIS.* *Dept. of Physiology, Univ. of Tennessee College of Medicine, Memphis.*

Following simultaneous intravenous injection of T-1824 and Na^{24} in the dog, rapid (2 to 3-second intervals) serial arterial blood sampling and analysis allows for the accurate construction of early 'disappearance' curves of both materials. Similar curves have been constructed for K^{42}. Plotting concentration of injected material per cc. of plasma divided by concentration injected per gram of dog against time, simultaneous comparisons will be made between a substance (T-1824) which disappears slowly from the vascular compartment, one (Na^{24}) which disappears at an intermediate rate and one (K^{42}) which disappears very rapidly. In such comparisons it is possible to differentiate the fractional contributions of hemodynamics and transcapillary exchange to the fate of injected substances. Such comparisons will be made in animals under the following experimental conditions: *1*) in hemorrhagic shock, *2*) during infusion of epinephrine solutions, *3*) following injections of hyaluronidase and *4*) during the hypotensive phase following spinal anesthesia.

Cardiovascular effects of serotonin, 5- and 7-hydroxytryptamine and tryptamine. IRVINE H. PAGE. *Research Div., Cleveland Clinic Fndn., and Frank E. Bunts Educational Inst., Cleveland, Ohio.*

Natural serotonin has the same cardiovascular actions as 5-hydroxytryptamine. 7-Hydroxytryptamine is qualitatively similar but quantitatively is less active than serotonin, but more active than tryptamine. In normal dogs without anesthesia or under pentobarbital, the tryptamines cause initial fall in arterial pressure—a von Bezold reflex—followed by a rise. In cats they are primarily depressor. Vasoconstriction occurs in perfused dog's legs and rabbit's ears, with a reflex vasodilator component when the legs maintain nervous connections with the body. Vagus nerve section or atropine abolish the initial von Bezold effect. The apnea followed by hyperpnea are not so influenced. Carotid sinus resection, spinal cord transection at C_6, ganglionic blockade with TEAC or hexamethonium—all augment responses. Peripheral blockade by benzodioxane and Priscoline do not abolish their action. 1-Hydrazino phthalazine accentuates the depressor action at the expense of the pressor. Cardiac output is probably increased. Tachyphylaxis is not observed. Adrenaline and noradrenaline are much more active as pressor substances but are not comparably active as vasoconstrictors in the vessels of the rabbit's ear. In neurogenic hypertensive dogs the pressor action may be reversed and the period of apnea prolonged. (The synthetic serotonin-creatinine was supplied by Dr. E. Hamlin, Jr. and Dr. R. K. Richards, Abbott Laboratories; and 7-hydroxytryptamine by Dr. Bernhard Witkop, National Heart Institute.)

Dextran, modified fluid gelatin and other plasma volume expanders in hemorrhaged dogs. W. M. PARKINS, J. H. PERLMUTT* AND H. M. VARS.

Harrison Dept. of Surgical Research, Schools of Medicine, Univ. of Pennsylvania, Philadelphia.

The oncotic and hemodynamic efficiency of several plasma volume expanders in the treatment of hemorrhagic shock have been assessed by two procedures: *1*) mild—immediate infusion after mean blood pressure declined to 20 mm. Hg; *2*) hypotensive—infusion after blood pressure has been maintained for 1 hour at 30–35 mm. Hg. Response to the various expanders, compared with that to autogenous blood, physiological saline and no treatment, was measured by: *1*) controlled bleeding volume determinations prior to infusion and at 3 and 24 hours postinfusion. The ratio of the volume withdrawn at each postinfusion interval to that removed during the initial hemorrhage has been designated the bleeding volume index (BVI); *2*) blood pressure and hematocrit; *3*) plasma level and excretion of the expander; and *4*) survival of the treated animals under conditions fatal to those untreated. Dextran (6%), oxypolygelatin (5 and 6%) and modified fluid gelatin (3%—Knox) were very effective in both procedures, though somewhat less so than whole blood; oxypolygelatin was inferior to the other two substances in the hypotensive procedure. Due to its increased oncotic activity, a 3% solution of the modified fluid gelatin (gel point: 0°–5°C.) was as effective as higher concentrations of dextran and other gelatins. Polyvinylpyrrolidone (3.5%), although producing hemodynamic reactions in the dog, is superior to saline, but less beneficial than dextran or the gelatins in this species. Modified human globin (4%), in its present form, with accompanying vasomotor reactions, was inferior to physiological saline. (This work was supported by contract number DA-49-007-MD-107 between the University of Pennsylvania and the Department of the Army.)

Crystalloid solutions as plasma volume expanders in hemorrhaged dogs. W. M. Parkins, J. H. Perlmutt* and R. O. Stait.* *Harrison Dept. of Surgical Research, Univ. of Pennsylvania, Schools of Medicine, Philadelphia.*

The comparative effectiveness of intravenous glucose, oral saline and intravenous saline has been measured by: *1*) bleeding volume determinations (4 cc/kg/min. to B.P. of 20 mm. Hg, followed by immediate infusion). The ratio of a second bleeding volume (H_2) at 3 hours and a third (H_3) at 24 hours postinfusion, to that of the initial (H_1), has been termed the bleeding volume index (BVI), *2*) blood pressure and hemodilution response, and *3*) survival. Values for the bleeding volume indices at 3 (H_2) and 24 (H_3) hours were: *A*) untreated dogs—H_2-17 and H_3-0; *B*) 5% glucose IV, 27 and 36; *C*) 5% glucose in 0.9% saline IV 47 and 43; *D*) 0.9% saline *1*) oral-1 volume (equal to volume of initial hemorrhage or 4–6% body weight) 16 and 16; *2*) 1 volume IV—54 and 68; *3*) 2 volumes IV (8–12% body weight) 65–83; *4*) 3 volumes IV (12–18% body weight)—73 and 65. Survivals were as follows: *1*) no treatment, 3 hr., 3 out of 6; 24 hr., none; *2*) oral saline—3 hr., 3 out of 5; 24 hr., 1; *3*) glucose—3 hr., 4 out of 5; 24 hr., 4; *4*) glucose in saline—3 hr., 6 out of 6; 24 hr., 4; *5*) 1, 2, or 3 volumes saline—3 hr., 5 out of 5; 24 hr., 5. Intravenous glucose provided the most favorable immediate response, but did not maintain the blood pressure and hemodilution as long as intravenous saline. Oral saline was definitely less effective than intravenous infusion in this situation where the time factor for absorption may be critical. Intravenous saline immediately infused in adequate amounts was effective in mild shock. Eight to 12% of body weight appeared to be the optimal volume, since larger amounts caused excessive hydration and gross edema. (This work was supported by contract number DA-49-007-Md-107 between the University of Pennsylvania and the Department of the Army.)

Acidity vs. acetylcholine and cation permeability of red cells. Arthur K. Parpart and Joseph F. Hoffman.* *Dept. of Biology, Princeton Univ., Princeton, N. J.*

Greig and coworkers (*Arch. Biochem.* (1949–51)) report that the active breakdown of acetylcholine by human red cells is correlated with a slower rate of loss of potassium from these cells. We have performed identical experiments on human red cells using several concentrations (10^{-2} to 10^{-4}) of acetic acid instead of acetylcholine and have obtained exactly the same results. We interpret these results as indicating that in the system studied the slowing of the rate of loss of potassium is due to the increased hydrogen ion concentration, produced either directly by addition of acid or by the breakdown of acetylcholine. Eserine added to the system causes no alteration of the effect obtained on adding acetic acid; though it does abolish the effect of acetylcholine. This is additional evidence for our interpretation. At present, therefore, there is no evidence for a relation between cation permeability of red cells and the activity of the acetylcholine-cholinesterase system.

Reflex latency changes associated with temporal summation. L. D. Partridge (introduced by C. R. Brassfield). *Physiology Lab., Univ. of Michigan, Ann Arbor.*

In study of ventral root potential changes resulting from repetitive stimulation of dorsal roots of isolate frog cords, it was noted, as previously reported, that each stimulus produced a relatively rapidly rising and slowly falling electrotonic potential. Repetition, even at low rates (less than

2/sec.), gave appreciable summation of the slow potential and increase of spike activity corresponding to degree of elevation of the slow potential above threshold strength. Closer inspection shows a pronounced reduction of latent period of spike activity to successive stimuli as slow potential levels approach threshold value. It is suggested that these data support the view that excitation is a function of slow potentials and thus offers simple explanation of variability of reflex latency.

Influence of dietary protein levels in starvation on the endocrine system. K. E. PASCHKIS, A. CANTAROW, J. RIVERO-FONTAN* AND E. WEST.* *Div. of Endocrine and Cancer Research, Jefferson Med. College, Philadelphia, Pa.*

In starvation the glands of internal secretion are reduced in weight and function. We have investigated the influence of different levels of protein intake under conditions of starvation. Two groups of starved rats received ¼ of the calories consumed by the controls. In one starvation group the protein intake was also reduced to ¼ of the control level; the second starvation group received 4 times as much protein, but the same total calories as did the first starvation group. The weight loss of pituitary and thyroid was identical in the 2 starvation groups. Diminished uptake of I^{131} (per mg. thyroid tissue) was identical in both starvation groups. There was no absolute weight loss of the adrenals. Weight loss of ovaries and testes was slightly, but not statistically significantly, greater in the low protein starvation group. Weight loss of the seminal vesicles and of the uterus was significantly greater in low protein starvation. In starved castrate male rats the level of protein did not influence the response of seminal vesicles and prostate to exogenous testosterone propionate. It appears probable that in starved animals on high protein diet the testis (and probably the ovary) secrete more sex steroids than on an equicaloric low protein diet. The weight loss of pituitary, thyroid and gonad was greater in females than in males, suggesting a greater sensitivity of the female pituitary to starvation. (Aided by a grant from the Ciba Corporation.)

Mechanisms of radiation protection of thymic cell suspensions. HARVEY M. PATT AND MARGARET E. BLACKFORD.* *Argonne Natl. Lab., Chicago, Ill.*

Sensitivity of rabbit thymocytes to x-irradiation *in vitro* is decreased by cysteine (22-hour LD_{50}: control 125 *r*, cysteine 250 *r*). Protection is dependent upon cysteine concentration, time of administration, oxygen and temperature. Minimal effective concentration is approximately 6×10^{-3} M; 3×10^{-3} M is ineffectual and maximal protection is observed above 12×10^{-3} M. These concentrations exceed 100 times the dose required to protect mice against acute radiation lethality (60 mg/gm. thymocytes and 0.5 mg/gm. mouse). Unlike the intact mouse, resistance of thymic suspensions is increased whether cysteine is added immediately before or after irradiation. Addition 15 to 30 minutes before exposure is optimal, however. Complete anoxia affords comparable protection to cysteine, but the two are not additive. Resistance of packed cells and failure of cysteine to affect them can be explained by anoxia. Chilling cell suspensions to 2°C. at the time cysteine is added or during the first half hour after irradiation diminishes protection. When cysteine is added more than 30 minutes before exposure, chilling during the early postirradiation period is without consequence, an effect indicative of a critical overall time of reaction. Results with anoxia suggest a similar temperature dependence. These findings cannot be ascribed to competition for toxic breakdown products of water nor are they a result simply of oxygen removal. Cysteine binds oxygen rapidly in thymic cell suspensions but the effects of chilling suggest that additional events occur. These data implicate a biological rather than a radiochemical site of action.

Studies with *p*-chloromercuribenzoate and x-radiation in mice. HARVEY M. PATT, SYLVIA MAYER* AND DOUGLAS E. SMITH. *Argonne Natl. Lab., Chicago, Ill.*

Since there is evidence that cysteine, glutathione and BAL diminish radiotoxicity by virtue of their sulfhydryl groups, the question arises as to whether toxicity will be modified by agents that inactivate such moieties; *p*-chloromercuribenzoate, which combines with but does not oxidize sulfhydryl groups, has been used for this purpose. Fifty mg/kg. i.v. kills about 60% of mice with a mean survival time of 3 days. Perhaps consistent with the idea that *p*-chloromercuribenzoate kills by binding sulfhydryl groups is the finding that lethality is largely prevented when cysteine is given either 10 minutes before or after mercuribenzoate. X-ray toxicity in mice (30-day survival) is not potentiated when a nontoxic dose of mercuribenzoate (15 mg/kg. i.v.) is administered either immediately before or after whole-body irradiation with 600 or 800 *r*. When toxic amounts of mercuribenzoate are injected (50 mg/kg. i.v.) lethality from the compound and the x-radiation appears to be additive. Postirradiation cysteine (1200 mg/kg. i.v.) can reverse the effect of a toxic dose of mercuribenzoate given prior to exposure to 600 or 800 *r* but radiation toxicity is unaltered under these conditions. These data fail to support the idea that inactivation of—SH enzymes is necessarily a critical factor in the development of radiation injury and that cysteine acts against x-radiation by protecting sulfhydryl groups.

Phylogenetic and functional evolution of human teeth. T. L. PATTERSON. *Dept. of Physiology, Wayne Univ. College of Medicine, Detroit, Mich.*

Previous gastrointestinal studies on sharks has focused attention on the relationship of tooth development from an integumental scale. It has also been observed that the irregularly placed teeth are arranged in several diminishing rows becoming continuous over the edges of the jaws with the scales of the skin. The teeth of the shark are triangular in shape, very sharp and are imbedded in a cartilaginous jaw. Furthermore, they show a decided slant in the caudal direction corresponding to that of the body scales. Histologically these scales are of the placoid type consisting of a flat, basal plate of dermal origin embedded in the skin and bearing on the top a very minute projecting point or spine tipped with enamel and resembling the structure of a human tooth. The primordial fishes from which modern vertebrates are descended were clothed with primitive projecting scales like their modern relatives, the sharks, skates and rays. Therefore, it is assumed, that, as animal life developed, a division of labor appeared among the descendents of the ancestral fishes in which the placoid scales lost the projecting spine but retained the basal plate and these became the scales of modern fishes, snakes and lizards. The scales located on the jaws, however, were used to hold and tear the animal's prey, resulting eventually in an enormous development of the projecting spines in comparison with the basal plate and in the course of time migrated into the mouth and became embedded in the cartilaginous jaw as teeth.

Responses in the corticospinal tract of cat and monkey. HARRY D. PATTON AND VAHE E. AMASSIAN.* *Dept. of Physiology and Biophysics, Univ. of Washington School of Medicine, Seattle.*

Electrical responses of the corticospinal tract to cortical shocks were studied in cats and monkeys under Dial or chloralose-tubocurarine. Recording microelectrodes were placed in the lateral columns of the cervical spinal cord. Single 0.1–5.0 msec. square wave shocks to the contralateral motor or sensory areas elicited complex responses. These consisted of a sharp positive deflection of 0.5–0.7 msec. latency (D wave) followed by a series of 1–4 later positive deflections (I waves) which were variable in appearance and latency. We believe D wave is derived from unrelayed corticospinal impulses and I waves by activation of cortical interneurons because *a*) the latency of D wave is accounted for by conduction time calculated from corticospinal fiber diameters; *b*) D wave can be elicited at rates corresponding to refractory periods of A fibers; *c*) D wave resists asphyxia and cocainization of the cortex far more than I waves; *d*) I waves are abolished by removal of cortex while D waves are elicited by stimulating exposed white matter. At given shock strength, the cortical area from which D wave is elicited is smaller than that yielding I waves so that movement of cortical electrode away from maximal point causes increased latency of discharge. From the region of pyramidal decussation (confirming Adrian and Moruzzi) single unit discharges of 4–8 msec. latency are readily obtained. However, these discharges are quite different from D wave in corticospinal tract (prolonged subnormality to a second cortical shock). This suggests that such discharges derive from at least second order neurons (probably bulboreticular).

Vascular patterns in ear as a source of error in oximetry. W. PAUL, B. G. GRAPES AND R. H. TAPLIN. (introduced by J. K. W. FERGUSON). *Dept. of Pharmacology, Univ. of Toronto, Toronto, Canada.*

A special earpiece was made with a removable photocell block replaceable by a lucite window. The window was marked to show the corresponding position of the two semicircular ('red' and 'infra-red') cell areas when in place over the illuminated pinna. Forty-one observations were made on subjects fully saturated by the administration of 99.5% oxygen. Photographs were taken of the illuminated area and saturation measurements were made with the oximeter, using it as an absolute reading instrument. On the basis of the appearance of dark vessels in one or other of the semicircles or on the apparent homogeneity of the area, the observations were divided into 3 groups. A statistical analysis was made and a significant correlation was shown to exist between the direction of the error from 100% and the presence of a vessel or vessels in the 'red' or 'infra-red' side of the illuminated area. Two special earpieces were constructed with a view to correcting the error in an inhomogeneous pinna, one with a lucite light pipe to scatter the transmitted light before allowing it to fall on the cells, the other similar to the standard earpiece except that the cell was divided into 8 sections rather than the 2 or 3 ordinarily used. A significant improvement in the standard deviation from 100% was achieved with both modified earpieces.

An anatomic dissection of dye dilution curves. M. L. PEARCE,* W. P. MCKEEVER,* P. DOW, M. MERRELL* AND E. V. NEWMAN. *Dept. of Medicine, Johns Hopkins Hosp., Baltimore, Md.*

When an indicator such as T-1824 is injected rapidly into the venous side of the circulatory system a time-dilution curve can be recorded from a systemic artery (Stewart-Hamilton). Cardiac output is calculated from the amount of dye injected and the area under the curve after correction for recirculation. If the successive intra-

thoracic volumes resemble serial compartments in which rapid dilution occurs then the logarithmic downslope of the time-dilution curve equals cardiac output divided by the largest single central volume. To test the validity of this serial compartment analogy, cardiac catheters were inserted into various parts of the central circulatory system of dogs, 0.4 to 1.5 mg. of T-1824 was then rapidly injected through the previously filled catheter. Dye dilution curves were recorded with a photomultiplier densimeter attached to a piece of polyethylene tubing which bridged the divided carotid artery, permitting many determinations on the same animal. Analysis of curves obtained from various injection sites indicates that the serial compartment analogy is valid and that the largest volume is between the pulmonary arteries and pulmonary veins. This is supported by the observation that curves resulting from injections anywhere between the superior vena cava and the pulmonary artery had similar downslopes, whereas curves resulting from injections between pulmonary veins and aorta had progressively steeper slopes. The volume derived from slope and flow has been consistently smaller in dogs than the one derived from the mean circulation time between pulmonary arteries and pulmonary veins.

Glycylglycine as a buffer in adenosinetriphosphatase assay. C. N. Peiss (introduced by F. E. Franke). *Johns Hopkins Univ., Baltimore, Md.*

Adenosinetriphosphatase (ATPase) assays were performed on homogenized whole brain and liver from adult albino rats. Incubations were carried out for 15 minutes in a water bath regulated at 37.5° ± 0.01°C. The gas phase was air. The optimum concentration of barbital buffer was determined separately for each tissue, and was identical (0.011 M) for both brain and liver ATPase. Similar experiments were done to establish the optimum concentration when glycylglycine was used as the buffer. Striking differences in behavior of the two tissues were noted. When glycylglycine concentration was varied over the range 0.0005–0.5 M, ATPase activity in brain was increased from 6.6–10.6 μg. P/mg. tissue/15 minutes. Above 0.5 M, further increases in activity appear in about 60% of the experiments. In the case of liver assays, varying the glycylglycine buffer concentration over the range 0.01 to 0.7 M decreased ATPase activity from 20.9 to 10.5 μg. P/mg. tissue/15 minutes. Decreasing buffer concentration from 0.01 to 0.001 M had no effect on the enzyme activity. Thus, in a glycylglycine buffer, rat brain ATPase activity increases with increasing buffer concentration; on the other hand, rat liver ATPase activity decreases with increasing buffer concentration. Comparison of ATPase activity at respective optimum concentrations of barbital and glycylglycine buffers was made for both brain and liver. ATPase activity is higher in glycylglycine buffer than in barbital buffer in both brain and liver by 34% and 43% respectively. (Aided by contract with the Office of Naval Research.)

Effect of skin temperature and hydrostatic pressure on evaporation through excised dog skin. C. N. Peiss and D. L. Davis (introduced by A. B. Hertzman). *Dept. of Physiology, St. Louis Univ. School of Medicine, St. Louis, Mo.*

Published data indicate that cutaneous evaporation from human subjects remains constant over an air humidity range of 17–80%. Maintenance of a constant evaporative rate under these conditions implies some mechanism whereby the vapor pressure gradient is kept constant. The concept of relative humidity of the skin has been proposed to explain these data. Recent experiments in this laboratory have investigated the physical factors determining the vapor pressure gradient in evaporation through excised dog skin. With constant environmental conditions, increasing skin temperature from 28° to 33°C. over a 6-hour period has no effect on evaporative rate. If the 5°C. temperature rise is accomplished over a 15-minute period, the evaporative rate rises greatly during the period of increasing temperature. If skin temperture is then maintained at this high level, evaporative rate declines over a period of several hours and returns to its initial level. These results are consistent with the hypothesis than skin humidity may vary with changing conditions, and implies that the amount of water in the skin available for diffusion is one factor contributing to the maintenance of a rather constant evaporative rate. The loss of water through excised dog skin, as well as human insensible perspiration, is thought to be a diffusion process. Our results indicate that varying the hydrostatic pressure from 1–13 cm. H_2O does not affect the evaporative rate in these experiments. (Aided by contract with the Air Materiel Command, Army Air Forces.)

Oxygen capacity, volume and water content of erythrocytes from the spleen of the dog. Kenneth E. Penrod. *Dept. of Physiology and Pharmacology, Duke Univ. School of Medicine, Durham, N. C.*

Several articles have appeared in the French literature in recent years which seem to indicate the structure and oxygen carrying ability of the red cells derived from the spleens of dogs are not identical with those of the active circulation. These differences have been viewed as important in forms of stress which bring about splenic contraction, such as hypoxia and asphyxia. A more direct approach than has heretofore been employed has been used to examine the nature of the splenic-stored red cells. Blood samples have been

obtained without stasis from the carotid artery and the splenic vein before and after epinephrine induced splenic contraction in anesthetized dogs. The oxygen capacity was determined in the Van Slyke apparatus, the relative cell volume in Wintrobe tubes and the water content of cells by ashing to constant weight. The following formula was then used to compute the amount of active hemoglobin in the red cell: Hematocrit × % dry weight × specific gravity × % Hb. in dry weight = active hemoglobin = oxygen capacity/1.36. Although the hematocrit was increased by one third after epinephrine the water content and cellular hemoglobin remained remarkably constant at 66% and 85%, respectively. Thus no evidence was found for any alteration in the structure of erythrocytes stored in the dog spleen but this technique affords no insight into a possible alteration in the hemoglobin-oxygen affinity of these cells.

Tests of pulmonary function utilizing recording oximeters. JOHN F. PERKINS, JR., WILLIAM E. ADAMS,* NICHOLAS CHRISTOFF,* ADOLFO FLORES* AND WILLARD W. WEBBER.* *Depts. of Physiology and Surgery, Univ. of Chicago, Chicago, Ill.*

During the past 2 years we have been using recording oximeters of the Millikan (relative reading) or Wood (absolute reading) type, assembled in our laboratories, in tests of pulmonary function. Our first test, the '*in vivo*, trans-pulmonary oxygen dissociation curve,' consists of plotting the partial pressure of oxygen in alveolar air against the per cent saturation of hemoglobin in arterial blood. Samples of alveolar air are continuously collected by Rahn alveolar sampler and are automatically analyzed by Pauling oxygen meter. Oxygen saturation is determined by oximeter. The patient is equilibrated with each of several oxygen-nitrogen mixtures ranging from 10–100% oxygen. The procedure requires less than 1 hour. In normal subjects the curve obtained approximates those reported by Riley using Haldane and Van Slyke analyses. In a patient with anatomical shunts between pulmonary arteries and veins the curve was depressed at its left portion but rose linearly at the right. The linear rise is attributed to oxygen dissolved in the plasma passing through normal lung elevating the saturation of the shunted blood. Curves on patients with emphysema, pneumoconiosis and certain carcinomas suggest physiological shunting, i.e. passage of blood through poorly aerated alveoli. It is hoped that this test, which has been carried out on over 70 patients, and on dogs with surgically produced pulmonary lesions of known severity, together with continuous records on patients of oxygen saturation during exercise, may facilitate evaluation of pulmonary function.

A method of measuring 1-hydrazinophthalazine in body fluids. H. MITCHELL PERRY (introduced by HENRY A. SCHROEDER). *Hypertension Div., Dept. of Internal Medicine, Washington Univ. School of Medicine, St. Louis, Mo.*

In order to learn something of the fate of 1-hydrazinophthalazine in hypertensive patients, a colorimetric method for its determination was devised, depending on a triketohydrindine complex. Apparently ninhydrin combines with the free hydrazine molecule which is vividly colored and strongly hydrophobic in acid. Despite a high chloroform-water partition coefficient, the dissociation constant limits sensitivity to approximately micromolar solutions. Our standard test system involves adding acetic acid to pH 3.0, making the ninhydrin concentration 0.012%, heating 7 minutes at 70°C., cooling, extracting with 0.1 volume chloroform, and reading on the Beckman spectrophotometer at 430 mmu. Preliminary extraction with chloroform was successfully tried on concentrated urine; however, it is unnecessary since no color has been developed in the absence of the drug. The procedure reveals that within 30 minutes of oral administration, urine concentration begins a gradual rise; apparently it does not reach its maximum for at least 4 or 5 hours and some excretion continues for 24 hours after a therapeutic dose. Total renal excretion of free or easily hydrolizable compound approximates 5% of that taken. The levels in protein-free blood filtrates approach the limit of the method. They begin to rise almost immediately after ingestion and become maximal 90–180 minutes later; thereafter they slowly decline to low values which probably persist for a day. Assuming absorption from the intestine one logically concludes that this drug is metabolized so that only a small fraction is excreted unchanged in urine.

A striking example of temporal summation of stimuli widely separated in time. D. C. PETERS* AND ROBERT GESELL. *Physiology Lab., Univ. of Michigan, Ann Arbor.*

Temporal summation of peripheral vagal inhibition of the heart was studied in the turtle. Supra maximal stimulation of the vagus was used to avoid spatial summation. Amplitude of auricular contractions served to register prevailing degree of inhibition. The effects of single shocks varied quantitatively with the individual preparation. In some instances a single shock would produce a gradual decrease of amplitude extending over several beats. In other preparations it produced immediate arrest succeeded by a progressively increasing auricular relaxation lasting as long as 40 seconds. These temporarily increasing inhibitory effects of a single shock gave way to characteristic recoveries in which amplitude in-

creased rapidly and then progressively more slowly as contractions approached normal strength. Temporal summation varied with frequency of stimulation and in exceptional instances was discernible with shocks separated by 2-minute intervals. The envelope of recovery contractions suggests that hydrolysis of acetylcholine, in conformance with the law of mass action, is importantly involved in the process of temporal summation of stimuli.

Effect of unilateral nitrogen breathing on pulmonary blood flow in dogs. RICHARD M. PETERS* AND ALBERT ROOS. *Lab. of Thoracic Physiology, Depts. of Surgery and Physiology, Washington Univ. School of Medicine, St. Louis, Mo.*

An immediate increase in pulmonary arterial pressure ascribed to constriction in the pulmonary vascular bed during breathing of gas mixtures low in oxygen was demonstrated by von Euler (in cats) and by Cournand (in man). In the rabbit unilateral nitrogen breathing resulted in a slow decrease in blood flow through the nitrogen breathing lung (Dirken and Heemstra). In the present investigation the acute effects upon pulmonary blood flow of unilateral nitrogen breathing during controlled respiration were studied in 12 dogs. The chest was opened and each lung ventilated by a separate Starling pump. During bilateral oxygen breathing flow through each lung and pulmonary and systemic pressures were measured. Either the right or left lung was then switched to nitrogen for periods up to 180 minutes. Cardiac output was determined and from left and right pulmonary venous and mixed arterial oxygen contents the ratio of flows to each lung computed on several occasions. In all but three animals a reduction of 14–68% of the original fraction of total flow to the nitrogen lung was found; they likewise showed a rise in pulmonary arterial pressure on 10% oxygen breathing. Prolongation beyond 25–70 minutes did not produce further reduction in flow. The changes in pulmonary artery pressure in relation to cardiac output will be discussed. A prompt return to control flow occurred after bilateral oxygen breathing.

Certain aspects of reflex and mechanical influences upon venous circulation. LYSLE H. PETERSON, *Dept. of Physiology, Med. School, Univ. of Pennsylvania, Philadelphia.*

One year ago data were presented which demonstrated qualitative changes in the tone of vein walls as a result of reflex, humoral and mechanical influences. The same method, as previously described, has been further tested and the data have been repeated and extended. It has been observed that the vena cava of intact anesthetized dogs are normally undistended. As the volume within the vessel increases suddenly there is a sharp increase in the P/V curve. This transition point is markedly reduced following parasympathetic stimulation indicating relaxation of venous tone and, conversely, a sharp increase following sympathetic stimulation and hemorrhage. The vein remains quite inelastic following the latter two conditions and markedly more elastic following parasympathetic stimulation. The P/V curves for the vena cava following death vary considerably, depending upon the state of the animal at the time of death. The magnitude of changes in P/V in the intact living animal under these circumstances was found to be 15 to 20 times. The state of the vein walls controls the fate of roughly $\frac{2}{3}$ of the blood volume. Considering the degree of change noted, the role of the venomotor system in the regulation of the circulation is important. A brief analysis of the effects of a square compression wave in collapsible tubes will be presented.

Technique of validating renal function studies when renal extractions are determined. R. A. PHILLIPS. *U. S. Naval Med. Research Unit No. 3, Cairo, Egypt.*

The recent appearance of several articles purporting to demonstrate anomalous glomerular and/or tubular function indicate the need for validation of the experimental data. The technique proposed requires, simply, that the reliability of each individual plasma analysis of substance X be verified by demonstrating that renal blood flow calculated from the plasma extraction of X and the hematocrit agrees with the flow calculated from the whole blood extraction of X within the limits of error in the analytical procedures employed. Data will be presented to demonstrate the usefulness of the technique in detecting diffusion of X from cells to plasma during the interval required for separation, by centrifugation, of plasma and cells of renal venous blood. The comparison of flows calculated from the extraction of 2 or more substances extends the usefulness of the technique. Applications, such as the time required for urine to reach the bladder, will be documented.

Estrogen-induced regression of coronary atherosclerosis in cholesterol-fed chicks. RUTH PICK (introduced by LOUIS N. KATZ). *Cardiovascular Dept., Med. Research Inst., Michael Reese Hosp., Chicago, Ill.*

Estrogen prophylactically inhibits coronary atherosclerosis in cholesterol-fed cockerels (*Circulation* 4: 468, 1951). The possibility was therefore suggested that estrogens may also be therapeutically effective against previously induced coronary atherosclerosis. To test this, 50 chicks were fed a mash supplemented with 2% cholesterol and 5% cottonseed oil for 5 weeks. Ten chicks sacrificed at that time had numerous, well-developed, lipid-laden, intimal plaques in the coronary arteries.

The remaining chicks were divided into 2 groups; both continued to receive the same cholesterol-supplemented diet. One group also received daily 1 mg. injections of estradiol benzoate. After 3 weeks of this combined regimen, autopsy revealed a paucity of coronary lesions, with little or no lipid demonstrable in the remaining plaques. In contrast, the other (control) group of cholesterol-fed cockerels, which received no injections, all showed numerous, extensive, lipid-laden coronary plaques. Examination of the lesions in the estrogen-treated birds revealed that the residual lipid is present in the media, adventitia and perivascular connective tissue, while the intima was lipid free. These findings indicate that estrogen not only prophylactically inhibits coronary atherogenesis but also induces regression of previously produced coronary lesions, despite continued feeding of the atherogenic stimulus (cholesterol).

Ascorbic acid-adrenal cortical relations to formed elements of blood. SAM J. PILIERO* AND ALBERT S. GORDON. *Dept. of Biology, Washington Square College, New York Univ., New York City.*

Adult male guinea pigs were divided into 4 dietary groups: ascorbic acid deficient (*ad lib.*), normal diet (*ad lib.*), paired-fed, and paired-weight controls. Reductions in red cell numbers and hemoglobin values occurred in the ascorbic acid-deficient animals by the 2nd week (early scurvy). Hemoconcentration observed in late scurvy (24–28 days) was also seen in the paired-weight controls. Blood and adrenal ascorbic acid concentrations decreased progressively up to the time of late scurvy. The hemic changes induced by ascorbic acid deficiency could be prevented by the administration of this vitamin. To determine the relation of the adrenal cortex to a possible influence of ascorbic acid on the blood elements, the effects of ACTH on the scorbutic and control animals were compared. In both these groups peripheral lymphopenia and neutrophilia were observed at 2, 4, and 6 hours after ACTH administration. Although a marked decrease in eosinophil numbers was noted by 6 hours after ACTH injection in both the early and late paired-weight controls, only a slight, statistically insignificant, drop occurred in the early and late scorbutic animals. Significant drops in adrenal ascorbic acid concentrations were noted in both the early scorbutic and paired-weight animals at 4 hours after ACTH administration. The extremely low adrenal ascorbic acid values in the late scorbutic animals were not influenced by ACTH. (Supported by a grant-in-aid from the American Cancer Society upon recommendation of the Committee on Growth of the National Research Council.)

Effect of rate of injection of alloxan on the development of diabetes in rabbits. I. J. PINCUS, J. J. HURWITZ* AND M. E. SCOTT.* *Dept. of Physiology, Jefferson Med. College, Philadelphia, Pa.*

Many workers have not appreciated that the role of the intravenous injection of alloxan may determine, in part, the mortality rate and occurrence of diabetes in surviving animals. The present study is reported because of an apparent lack of information on this aspect of alloxan administration. Alloxan monohydrate was injected intravenously as a 5% solution into the marginal ear vein of rabbits at varying rates. Animals with persistent hyperglycemia and glycosurea were classified as diabetic, those with inconstant hyperglycemia or with an abnormal glucose tolerance curve were classified as mild diabetic. Eleven rabbits received 75 mg/kg. in 11–35 seconds; seven died and of the remaining four, two had diabetes. Ten rabbits received a similar dose in 35–100 minutes; one died and only two of the survivors had mild diabetes. 100 mg/kg. of alloxan was administered to 11 rabbits in 9–35 seconds; six died, four had diabetes and one mild diabetes. Of eight rabbits receiving the same dosage in 22–50 minutes one died, three were diabetic and one had mild diabetes. 150 mg/kg. of alloxan was injected intravenously into 66 rabbits in 5–60 seconds. Thirty-nine died and of the surviving 27 animals 15 had diabetes, seven had mild diabetes and five were not diabetic. Twelve animals received 150 mg/kg. in 5–18 minutes; three died and all of the nine surviving rabbits had diabetes. Sixteen rabbits received the same dosage in 30–50 minutes; two died and five had mild diabetes.

Effect of mercurial diuretics on excretion of water. R. F. PITTS, D. R. AXELROD, W. S. WIGGINS AND J. N. CAPPS. *Dept. of Physiology, Cornell Univ. Med. College, New York City.*

The mechanism of action of mercurial diuretics on the excretion of water has been studied in man and dog. Following the induction of a large water load and the administration of as much as 8 mg. Hg/kg. as mercurin in the dog (1 mg. Hg/kg. in man), the infusion of 100 to 250 mU of Pitressin/hour promptly reduced urine flow and caused the urine to become hypertonic to the plasma. We conclude from these results that mercurial diuretics do not directly interfere with active distal tubular (facultative) absorption of water, i.e. that moiety regulated by Pitressin. In 8 of 10 experiments on dogs and in 3 of 6 experiments on man subjected to a water loading procedure calculated to establish and maintain essentially maximal water diuresis, the administration of similar doses of mercurial diuretics did not increase urine flow above the high levels established in the control periods. These experiments suggest that mercury does not affect the so-called obligatory absorption of water,

namely that moiety passively absorbed in the proximal tubule in consequence of the absorption of osmotically active solutes. Contrary results, i.e. increased urine flow in a small minority of the dog experiments and in half of the experiments on man, have been tentatively assigned to fairly sharp and significant increases in glomerular filtration rate.

Sources and sinks of current in the spinal cord. W. Pitts, P. Wall and J. Lettvin (introduced by Mary A. B. Brazier). *Massachusetts Inst. of Technology, Cambridge and Dept. of Anatomy, Univ. of Chicago, Manteno State Hosp., Chicago, Ill.*

We have inserted from 9 to 18 microelectrodes in a transverse plane through segments L: and S: of the cat's spinal cord. Having taken records after various kinds of stimulation, constant in any one experiment, at many stations along the tracks of the microelectrodes, we leave them in place and determine their positions by fixing and clearing the cord. The result is a set of potential histories at precisely known places forming a dense network across a segment of the cord. At each time, we calculate the Laplacian of the distribution of the potential in space by numerical differentiation, thus obtaining a map of the sources and sinks of current across the cord. A source of current measures how much current flows out of cells and fibers into the external medium there and then; a sink, how much flows in. Consequently it is sources and sinks, rather than potentials, that represent the activity of neurons where they are. We present a series of maps, showing how sources and sinks are generated, move, and decay with time after an external stimulus. In some maps, large and rapidly changing potentials are present everywhere, without any significant sources or sinks in the region covered; they are generated some distance away. We conclude that little can be inferred, in general, from records taken with one or a few microelectrodes, as to the origin of the potentials they record; only a dense network of them permits us to localize the active elements.

Rate of change in concentration of serum magnesium upon cooling and rewarming. Wesley S. Platner. *Dept. of Physiology and Pharmacology, Univ. of Missouri School of Medicine, Columbia.*

It is now generally accepted that a fall in body temperature is responsible for a rise in serum magnesium. Exposure of turtles to environmental temperature of 3.0°C. ± 1°C. results in a body temperature fall which reaches environmental temperature in approximately 2½ hours. Serum Mg, as determined by a modified Titan yellow method, rises during this time from 3.2 to 6–8 Mg % and reaches a peak after 2 to 6 hours of exposure. Continued exposure shows a decrease in serum Mg so that after 17–20 days the Mg level returns to near normal. Rewarming the animals to room temperature 26.0 ± 2°C. causes a progressive serum Mg decline not directly correlated to changes in body temperature. This decline proceeds linearly at an approximate rate of 0.006 mg.%/minute. At this rate normal Mg values at room temperature are reached after 20–30 hours, depending on the initial concentration of Mg and exposure time to cold. Preliminary determinations of serum sodium and potassium by internal flame-Photometry did not show these changes.

Concentration and potential pattern within the membrane and its relation to penetration of ions and to time constants of electrotonus and accomodation. M. J. Polissar (introduced by F. H. Johnson). *Biomechanics Group, Univ. of California, San Francisco.*

The functional relation between a variable and the positional coordinate is referred to as its *profile*. A linear potential profile within a membrane is a rare exception. If the net rate of outward movement of an ion is R, the equation: $R = P(C_i - C_o)$ yields misleading values of P. The equation: $R = P(C_i - C_o^{\pm FE/RT})$, for univalent ions, is more appropriate (E is the total transmembrane potential). The rate is a function of the detailed profile of the potential, and, even with fixed values of C_i, C_o, E, it may vary by powers of 10 if the profile is changed. If the potential at a point of the outside surface is changed, by means of an electrode, the original steady-state pattern of profiles of potential and of concentrations changes to a new pattern. The time constant of this process must play a significant role in the physical interpretation of the time constants of electrotonus and accomodation. The finite rate of transition from one pattern to another may be used to interpret supernormal irritability at cathode make, accomodation with slowly rising currents, subnormal irritability at cathode on break, the opposite phenomena at the anode, including excitation at the anode at break.

Hypothalamic involvement in stress-induced eosinopenia. R. W. Porter (introduced by H. W. Magoun). *Dept. of Anatomy, Univ. of California School of Medicine, Los Angeles, and Long Beach Veterans Administration Hosp., Long Beach.*

The role of the hypothalamus in the pituitary-adrenal response to stress was studied in cats by: *1*) recording alterations in hypothalamic electrical activity in response to stress stimuli; *2*) determining the eosinophil response to direct hypothalamic stimulation; *3*) comparing the eosinopenia in-

duced by stressing agents before and after making electrolytic lesions in the hypothalamus. The findings were: *1)* A marked increase in the motor frequency and amplitude of electrical activity in response to intravenous epinephrine was localized to the posterior hypothalamic area at the mammillary level. Such stress-evoked hypothalamic discharge was uninfluenced by decortication or transection of the lower brain stem. *2)* In acute stimulation experiments, animals were prepared under cyclopropane and local procaine anesthesia without alteration of the normal level of circulating eosinophils. Direct stimulation of the posterior hypothalamus then produced an eosinopenia. *3)* In chronic animals destruction of the posterior hypothalamic area abolished the eosinopenic response to epinephrine and other stress stimuli seen before operation. Results from all three approaches thus indicate the primary participation of the posterior hypothalamus in the eosinopenia evoked by stress.

Strength-duration curves of single muscle fibers. JULIUS PRAGLIN (introduced by C. LADD PROSSER). *Dept. of Physiology, Univ. of Illinois, Urbana.*

The strength duration curves of single striated frog muscle fibers were investigated with square-wave stimuli in the range of 30 microseconds to 1000 milliseconds. The responses of single fibers were recorded electrically in oil by means of microelectrodes from the surface of the muscle. Mechanical responses were observed in single uninjured fibers isolated from the semi-tendinosus muscle and stimulated in solution. Strength-duration curves of fibers stimulated in solution show a curve which displays an α and γ branch. Under curarization the γ branch disappears and the excitation constant (Hill's K) of the α branch shortens from about 10 to about 3 msec. The γ branch has a K value of about 0.3 msec. This is identical with the motor nerve excitation constant. Stimulation under oil with small electrodes (0.13 mm. in diameter) yields an excitation constant for the muscle of 0.3 msec., which is identical to the motor nerve constant. Curare does not change this value significantly nor does it abolish a further discontinuity which occurs at very short durations (60–80 microseconds). This may be identical with Lucas' beta-excitability and has a K value of 30–50 microseconds. The accommodation curve (direct measurement in oil) shows a minimum in the region of the strength-duration curve discontinuity. Finally it has been shown that muscle strength-duration curves fit Hill's or Blair's one factor theory unequivocally; that is all curves plotted by Blair's (*Biol. Symp.* 3: 51, 1951) formula have zero intercept at the $\ln \frac{V}{V - R}$ axis.

Interrelations of pressures of gut lumen, abdominal cavity and thoracic cavity. J. P. QUIGLEY, D. A. BRODY* AND J. M. HUDGINS.* *Div. of Physiology, Univ. of Tennessee, Memphis.*

Pressures were recorded for the first time accurately, continuously and simultaneously from the thoracic cavity, the lumen of the gut and from the immediately adjacent portion of the abdominal cavity of unanesthetized, trained dogs. Additional unique features included the conduction of these pressures from each space by fluid transmission to appropriate calibrated strain gauges and the exact position was ascertained of the recording tip in the abdominal cavity. The influence of intrathoracic pressure on intraabdominal pressure and of intraabdominal pressure on gut intraluminal pressures under a variety of conditions was investigated by both the theoretical and the experimental method. (Supported in part by a research grant from the U. S. Public Health Service.)

Pyloric sphincter activity in normal dog. J. P. QUIGLEY AND HORTENSE LOUCKES.* *Div. of Physiology, Univ. of Tennessee, Memphis.*

Pyloric sphincter activity was recorded by the inductographic technic of Quigley, Louckes and Brody in unanesthetized, trained dogs. The sphincter characteristically contracts rhythmically, especially following feeding. The sphincter strongly tends to follow a constant pattern in the individual animal. The cycles average 11 seconds (range 8.5–13) and are not readily modified by experimental procedures. Ingestion of food by the fasting animal lengthens the cycle by 1–2 seconds within approximately 10 minutes. Noxious stimuli (slapping) or emotional disturbances suppress 1–4 cycles. The 'effective' contraction and 'effective' relaxation of the sphincter are abrupt and together involve about 45% of each cycle. The 'semi-rest' period constitutes approximately 65% of the cycle and is shortened as the cycle shortens. During the semi-rest period, the sphincter is slowly contracting, slowly relaxing or is quiescent. The amplitude of a sphincter contraction as obtained by measuring the intercoil distance is 0.4–1 cm. Usually the amplitude is greater and more uniform following feeding due to a greater relaxation (tonus loss) and also greater contraction. Contraction amplitude is decreased and made more irregular by noxious stimuli or emotions. The amplitude tends to be very irregular in the upset or ill-trained animal. Sphincter relaxed length during fasting usually was increased (decreased tonus) by 4–15% 60–90 minutes after feeding. Increased tonus suggesting pylorospasm occurred rarely and under unusual conditions such as vomiting. (Supported in part by a research grant from the U. S. Public Health Service.)

Fate of other pituitary hormones during purification of corticotropin. M. S. RABEN,* I. N. ROSENBERG* AND E. B. ASTWOOD. *Dept. of Medicine, Tufts College Med. School, and New England Center Hosp., Boston, Mass.*

The glacial acetic acid method efficiently removed corticotropin, growth hormone, luteotropin, intermedin, and adipokinin(s) in 20% of the solids from pig pituitary powder; thyrotropin and gonadotropins were partly destroyed or incompletely extracted. One-half volume of acetone and 0.02M NaCl precipitated one-quarter of the solids containing virtually all the thyrotropin and gonadotropins, most of the luteotropin, and variable small quantities of corticotropin. One volume of ether then precipitated one-half of the solids with the corticotropin, growth hormone, and intermedin. The filtrate was free of these factors. Solution of the ether precipitate in 0.1N acetic acid, stirring with 8% by weight of oxycellulose and elution of the adsorbed material with 0.1N hydrochloric acid, yielded 2% of the solids, virtually all of the corticotropin, and about one-half of the intermedin. Residual corticotropin and intermedin were recovered by retreatment with 20% by weight of oxycellulose. The residue contained growth hormone which was purified and freed of other factors by a modification of Wilhelmi's procedure. Separation of intermedin from corticotropin by readsorption of the latter on a smaller quantity of oxycellulose or by partition between various acids and butanol (intermedin the more hydrophilic) yielded preparations of these two factors in good yield and roughly 400 times as concentrated as the starting material. The adipokinetic effect was exhibited by several fractions; the posterior lobe principles though extracted did not contaminate the corticotropin, intermedin, or growth hormone.

Effects of diet selection and fasting on hypertensive rats. EDWARD P. RADFORD, JR. (introduced by E. M. LANDIS). *Dept. of Physiology, Harvard Med. School, Boston, Mass.*

Male and female normal and hypertensive rats were offered casein, brewers' yeast, olive oil, cod-liver oil, sucrose, distilled water, 0.2M NaCl, and a salt mixture in order to detect differences in appetite for these substances and effects of diet on systolic blood pressure. These particular foods were used to permit comparison with the intakes observed by Richter in normal and depancreatectomized rats offered the same choices. In confirmation of previous results both male and female hypertensive rats took less sodium chloride solution than the controls. Female hypertensive rats ingested significantly less of the protein foods (casein and yeast) and lost weight for an initial period of 1 to 2 weeks; subsequently, their protein and caloric intake approached that of the normotensives. Food selections of male hypertensive rats were not significantly different from those of the controls. Changes in systolic blood pressure were small and were also apparently related to sex. This sex difference assumes greater importance because latex encapsulation of the kidneys produces a more severe and progressive hypertension in female rats than in males. Not only do the females tend to develop higher systolic pressures but they also do not survive the complications of hypertensive disease as long. Hypertensive and control rats were also fasted until death while offered only water, 0.2M NaCl and 0.2M KCl. The hypertensive rats died after 2–7 days (mean 5 days) while the control rats died after 10–18 days (mean 14 days). Hypertensive rats lost 30.3% of their initial weight; normal rats 43.1%, although per diem weight loss by hypertensive rats was twice as great as that by normal rats. Systolic blood pressures of hypertensive rats remained elevated; those of normal rats rose slightly as bradycardia developed.

Some effects of plasma volume expanders on renal function. LAWRENCE G. RAISZ (introduced by H. W. SMITH). *Surgical Research Unit, Brooke Army Hosp., Ft. Sam Houston, Tex.*

The renal responses to rapid intravenous infusions of 20 cc/kg. of body weight of dextran (6%), oxypolygelatin (5%), human albumin (4–5%) and isotonic NaCl solution have been compared in the same normal dogs with and without preloading with oral saline. Glomerular filtration rate (GFR), effective renal plasma flow (ERPF), Na and K excretion, plasma volume (using T-1824) and the plasma concentration of colloids and electrolytes were measured. Dextran caused the greatest plasma volume expansion and saline caused the least, while albumin and oxypolygelatin were intermediate. GFR was increased to a similar extent with all the materials infused, this response being maintained throughout the 3-hour period of observation. ERPF was greatly increased after albumin infusion while with the other materials smaller increases occurred which were usually directly correlated with plasma volume expansion. ERPF reached a peak 1 to 2 hours after infusion and then fell gradually. Sodium and water excretion increased 2- to 4-fold within 1 hour after infusion, while potassium excretion peaked at the same time or shortly thereafter. The magnitude of these increases did not depend on plasma volume expansion, being as great after saline as after colloid infusions. In preliminary experiments infusions of washed red cells have not caused such increases in renal function and electrolyte excretion. The relative effects of interstitial fluid, plasma volume and blood volume changes on

renal hemodynamics and electrolyte excretion will be discussed.

Rate of atrophy in muscles immobilized at different lengths. H. J. RALSTON, B. FEINSTEIN* AND V. T. INMAN.* *College of Physicians and Surgeons, San Francisco, and Univ. of California Med. School, San Francisco.*

It is generally recognized that muscles immobilized at short lengths exhibit atrophic changes within 15–30 days. The present study was undertaken to throw more light on the protective action of stretch in immobilized muscles, as claimed by some authors. Twenty-eight muscles were studied in 17 guinea pigs, for periods of immobilization ranging from 31 to 65 days. Limbs were placed in cork casts, in positions of extension or flexion, care being taken to allow normal circulation to the muscles. In 16 cases of extension, the experimental muscles weighed 87%–117% of the controls, at the end of the period of immobilization. Functional behavior of the muscles was normal, as measured by the length-tension diagram. Muscles immobilized at short lengths in all cases lost weight, ranging from 72%–95% of controls, confirming earlier studies. It is concluded that stretch protects against atrophy in immobilized muscles, agreeing with common clinical observation in the human subject. (This study was supported by a grant from the National Foundation for Infantile Paralysis.)

Sweating patterns on the lower extremity of man elicited by stimulation of the sympathetic trunk. W. C. RANDALL, W. F. ALEXANDER,* K. E. COLDWATER,* A. F. HERTZMAN AND J. W. COX.* *Physiology Labs., St. Louis Univ. and Surgical Service, Veterans Administration Hosp., Jefferson Barracks, St. Louis, Mo.*

In a direct approach to the problem of the sympathetic innervation of the lower extremity in man, sweating patterns have been recorded simultaneously from the circumference of the thigh, leg and foot by the iodine-starch paper technique during electrical stimulation of the lumbar sympathetic trunk and its communicating rami. The sympathetic trunk was removed and prepared for careful microscopic study in order to correlate the functional responses with the anatomical pathways into and out of the ganglion chain. Marked variation in the patterns of sweating in different patients to stimulation at any given level on the sympathetic trunk emphasizes the fact that pre- and postganglionic pathways to the extremities are markedly inconstant. In some patients, stimulation of the sympathetic trunk at the vertebral level between T_{12} and L_1 induced profuse sweating over most of the lower extremity. In others, such stimulation activated sweat glands only on the thigh while sweating on the foot did not appear until the stimulation was applied as low as L_4 or below. This implies that preganglionics mediating sweating responses may enter the trunk at widely varying levels between these extremes, and that surgical removal of the uppermost ganglia does not insure certain denervation. Disparity between sudomotor and pilomotor responses to stimulation was frequently observed and therefore it may not be valid to infer vasomotor fiber distribution from observations of sudomotor or pilomotor responses.

Actomyosin content of dog myocardium. R. E. RANNEY (introduced by CLARENCE A. MAASKE). *Dept. of Physiology and Pharmacology, Univ. of Colorado School of Medicine, Denver.*

Samples of the myocardium and of the psoas muscle were removed by a standardized procedure from anesthetized dogs and cooled slowly to −15°C. The frozen muscle tissue was homogenized, and the actomyosin was quantitatively extracted. It was found that about 52% of the total N in the psoas was contributed by actomyosin. Of the total N present in left ventricular muscle 42% was present as actomyosin N; in the right ventricle this value was 37%; and the auricles contained equal amounts of actomyosin N, about 16% of the total. It should be noted that since the auricles contain proportionately more connective and elastic tissues than do either the ventricles or the psoas, the amount of auricular actomyosin given above is probably less than that actually present in the muscle alone. The distribution of actomyosin in dog myocardium agrees qualitatively with that reported by Kovats (*Nature, London* 163: 606, 1949) for the pig and the rabbit. It is apparent from these data that the concentration of actomyosin is not constant throughout the dog myocardium, and, furthermore, the possibility is suggested that the amount of work done by the musculature of any chamber of the heart may be roughly correlated to its total actomyosin concentration.

Renin and epinephrine responses after severe hemorrhage. G. CARL RAU AND FERRILL A. PURDY.* *Dept. of Physiology and Pharmacology, Univ. of Missouri School of Medicine, Columbia.*

In the regimen of studying pressor responses of animals receiving various compounds after severe hemorrhage, and the relationships of the sympathetic nervous system and the kidneys to the ability to compensate for blood loss, the comparative responses of renin and commercial epinephrine were particularly noted in an attempt to clarify differences of opinion on the effects of these two compounds. Thirty-two dogs of the same sex and similar weight were used, 16 animals were given 10 injections of epinephrine and 16 were given 10 injections of renin. Dogs receiving epinephrine

showed no tachyphylaxis while those receiving renin gave the typical tachyphylactic picture. The dogs were subsequently hemorrhaged to 40% of the total blood volume and half of the animals were injected immediately with epinephrine while the others were given renin. All injections were made i.v. using posthemorrhage mean blood pressure change as a measure. Animals injected with renin gave a much greater and sustained pressor response than before hemorrhage, while all except two of the dogs receiving epinephrine gave a smaller and less sustained pressor response. Results indicate that renin functions much more efficiently than epinephrine in counteracting posthemorrhage hypotension. Renin for these experiments was kindly supplied by Dr. O. M. Helmer, Lilly Laboratories for Clinical Research.

Bioelectrical patterns in the synovialis of the dog. C. I. REED. *Dept. of Physiology, Univ. of Illinois, Professional Colleges, Chicago.*

Explanation of the behavior of synovial bioelectric potentials in dogs and in arthritic patients previously reported, requires first a systematic survey of patterns of potentials observed in normal dogs. Technic has been described previously. Initial potentials observed simultaneously in the two knees diverged in 83% of animals, quantitatively in 51%, qualitatively in 22%. Repeated washing with Ringer solution eliminated the differences in 62% of all animals. Adenine eliminated them in an additional 13%. In the remainder, quantitative differences persisted indefinitely. It seems unlikely that the observed potentials are dependent upon systemic metabolic factors. Blocking circulation and nerve supply to the joint did not regularly alter potentials induced by adenine.

Critical study of some physiological factors responsible for differences in estimates of total blood volume given by plasma volume and plasmacrit and by cell volume and hematocrit determinations. E. B. REEVE,* M. I. GREGERSEN, T. H. ALLEN AND H. SEAR.* *Dept. of Physiology, College of Physicians and Surgeons, Columbia Univ., New York City.*

Estimates of total blood volume derived from either a measurement of cell volume and the hematocrit (B.V. = cell volume × 100/hematocrit) or from a measurement of plasma volume and plasmacrit (BV = plasma volume × 100/plasmacrit) may or may not agree with the sum of cell and plasma volume ('true total blood volume'). We have simultaneously determined cell volume with ^{32}P and plasma volume with T-1824. In normal unanesthetized dogs the ratio ^{32}P blood volume/'true blood volume' ($R^{32}P$) ranged from 0.96 to 1.07, average 1.0, and the ratio T-1824 blood volume/'true blood volume' (*R T-1824*) from 0.95 to 1.03, average 1.0. In two dogs under Nembutal anesthesia, which dilates the spleen, $R^{32}P$ was initially 1.1 and 1.04 and *R T-1824* was 0.95 and 0.97. On injecting adrenaline to contract the spleen $R^{32}P$ fell to 0.87 and 0.83, respectively, and *R T-1824* rose to 1.09 and 1.12. The same changes were observed after adrenaline in some normal unanesthetized dogs. The importance of the spleen was further demonstrated by the fact that in unanesthetized splenectomized dogs $R^{32}P$ ranged from 0.89 to 0.93, average 0.91, and *R T-1824* from 1.05 to 1.1, average 1.07. The effects on these ratios of drastic changes in blood volume and circulation have also been investigated. (Work supported by a grant from the Lilly Research Laboratories.)

Electrical resistance of the stomach. WARREN S. REHM AND HILDE SCHLESINGER.* *Dept. of Physiology, Univ. of Louisville, Louisville, Ky.*

In previous work (*Am. J. Physiol.* 163: 744, 1950) it was found that clamping the pedicle to a portion of the anesthetized dog's stomach, placed in a lucite chamber and in contact with saline, resulted in an increase in its electrical resistance. In the present experiments an attempt was made to determine the site of this increase in resistance. Electrical resistance was measured by sending small direct currents across the stomach via one pair of electrodes and measuring the P.D. before, during, and after the sending of the current via a second pair of electrodes. It was found that clamping the pedicle while isotonic hydrochloric acid was in contact with the mucosa resulted in a much smaller increase in resistance than when saline was in contact with the mucosa. The resistance reached a peak and started decreasing much sooner with hydrochloric acid than with saline. When the pedicle was clamped and the resistance had increased considerably, it was found that replacing the saline with hydrochloric acid on the mucosal side resulted in a very marked drop in resistance within two minutes. Bubbling oxygen through the saline on the mucosal side resulted in a decrease in the resistance when the pedicle had been clamped previously, and a marked delay in the increase in resistance when the pedicle was clamped after the oxygen had been started. The effect of nitrogen was opposite to that of oxygen. These results support the hypothesis that the surface epithelial cells are the site of the increased resistance.

Effect of acute vascular occlusion on thoracic duct lymph flow. W. O. REINHARDT AND M. GEISENDORFER.* *Div. of Anatomy, Univ. of California School of Medicine, Berkeley.*

Unfasted adult male rats under sodium pentobarbital anesthesia were subjected to cannulation of the thoracic duct at its entrance into the left jugulosubclavian angle. After a 30-minute con-

trol period, lymph flow was measured for a 1-hour period following immediate occlusion of various main vascular trunks. During the 1-hour experimental period, lymph flow for 11 control animals averaged 0.60 ± 0.07 ml.; for 10 rats with ligation of the hepatic portal vein the lymph flow was 2.08 ± 0.35; and for 7 rats with ligation of the superior mesenteric vein, lymph flow was 4.35 ± 0.38 ml/hr. Ligation of the suprahepatic inferior vena cava reduced lymph flow to 0.15 ± 0.06 ml., and combined ligation of the portal vein and hepatic artery in 9 rats produced a lymph flow of 1.07 ± 0.16 ml. The severity of the vascular occlusions was measured by fatalities within a 2-hour period in the groups with occlusion of the suprahepatic inferior vena cava or with combined ligation of the portal vein and hepatic artery, whereas occlusion of the portal vein or of the superior vena cava was not uniformly fatal in a 2-hour period of observation. It would appear from these results that maintenance of a partial hepatic circulation, arterial or venous, is essential to the production of a continued increased lymph flow from the splanchnic area after portal or systemic vein occlusion, and that occlusion of a tributary of the portal vein (superior mesenteric vein) is, for that reason, more effective in altering lymph flow than is ligation of the portal trunk itself. Whether the effects described are dependent on the total hemodynamic picture or on a specific role of the liver in altering capillary permeability, directly or indirectly, can only be made the subject of further experimentation.

Measurement of bovine plasma volume with T-1824 and estimation of the blood volume. MONICA REYNOLDS (introduced by F. H. MCCUTCHEON). *School of Veterinary Medicine, Univ. of Pennsylvania, Philadelphia.*

The T-1824-dilution method has been adapted and used for measuring plasma volumes in healthy, nonlactating Guernsey cows. Spectral absorption curves of dye at various concentrations in cow serum are similar to those in dog or human serum. Maximum absorption occurs at 620 mμ, where the concentrations conform to the Beer-Lambert law throughout a density range of 0.4–1.2. A density value of 0.84 ± 0.01 was found for a 1.00-mg.% solution of dye in bovine serum. Following an injection of dye, changes in concentration during the first hour yield a curve similar in contour to those obtained with other species. Complete mixing appears to occur in less than 15 minutes. In one cow, 300 kg. average weight, 11 plasma volume measurements were made at intervals during a 3-month period. Results ranged from 11.3–12.5 liters and averaged 11.8 ± 0.3 liters. Since the estimated probable error in the method is 3% this 5% variation represents to some extent small but real changes in plasma volume. Eleven blood volumes on the same cow, calculated from uncorrected hematocrit values, ranged from 16.3–18.2 liters and averaged 17.0 ± 0.5 liters. Plasma and blood volumes measured on 15 different cows averaged 39 ± 1.2 cc/kg. and 60 ± 3 cc/kg., respectively. These values are shown to be consistent with those obtained in other mammals when the same method is used.

Maximal diffusing capacity of lungs. RICHARD L. RILEY. *Dept. of Environment Medicine, Johns Hopkins Univ., Baltimore, Md.*

The diffusing capacity is primarily a function of the diffusion characteristics of the surface across which gas exchange takes place and of the total area of this surface. When estimated by the method recently described by Riley, Cournand and Donald, the diffusing capacity is found to increase sharply during exercise. This phenomenon suggests that the diffusing surface increases by an increase in the diameter of the pulmonary capillaries and/or by an increase in the number of capillaries across whose walls gas exchange takes place. For healthy young men the maximal diffusing capacity is found to be approximately 40 ml/min/mm. Hg gradient/m^2 body surface. Experimental conditions are defined under which maximal or near maximal values for diffusing capacity can be obtained. Since under the specified conditions gas exchange presumably occurs across all available capillaries, the maximal diffusing capacity is thought to provide an *in vivo* estimate of the size of the pulmonary capillary bed.

Contraction of heart muscle. JANE SANDS ROBB. *Dept. of Pharmacology, New York State College of Medicine, Syracuse, N. Y.*

The coronary bed of untreated hearts of small mammals has been perfused with oxygenated Tyrode solution. Pressure and temperature were controlled, the cavities were not allowed to fill. Simultaneous electrocardiograms and contraction waves were recorded, the latter by means of a transducer and strain gage amplifier which replaced one unit of a Sanborn Polyviso Recorder. Arrhythmias were sometimes spontaneous, at other times induced by a stimulator which could deliver square waves of various frequencies, durations and strengths. No heart failed to demonstrate summation and incomplete tetanus although more patience was necessary to demonstrate these phenomena at 39° than at 20°C. Response at high temperatures, with this preparation, is unlike that reported in 1951 by DiPalma and Mascatello who used a papillary muscle preparation. At least six other investigators have seen summation and tetanus in heart muscle but unusual circumstances served to deemphasize

their observations. Newer concepts of myocardial structure make a similarity of cardiac and skeletal muscle response seem the rule rather than the exception.

Effects of cortisone administration on renal tubular capacity for reabsorption of phosphate and excretion of potassium and titratable acid. KATHLEEN E. ROBERTS (introduced by JOHN MACLEOD). *Dept. of Physiology, Cornell Univ. Medical College, New York City.*

The maximum renal tubular reabsorptive capacity for phosphate has been determined in normal and in adrenalectomized dogs both before and after the administration of cortisone. It has been found that the administration of this steroid either previous to phosphate loading or during the infusion of phosphate results in a depression of the tubular reabsorption of phosphate both in the intact and in the adrenalectomized dog. When the amount of filtered phosphate was increased above the renal tubular capacity for reabsorption, increasing amounts of phosphate were excreted; this was accompanied by a marked rise in the urinary excretion of titratable acid and potassium. Concomitantly with the increase in potassium excretion, there was a marked fall in the plasma potassium level. This increase in urinary potassium and titratable acid excretion, as well as the fall of the plasma K^+ level was observed both in the control and in the cortisone-treated animals and could be correlated with the amount of phosphate excreted. Experiments are now in progress to compare the effects of desoxycorticosterone and cortisone on the phosphate reabsorptive capacity, as well as potassium and titratable acid excretion in the intact and in the adrenalectomized animal. (Financed by a grant from the American Heart Association.)

Basal metabolism of the Eskimo. KAARE RODAHL (introduced by A. P. GAGGE). *Arctic Aeromed. Lab., Ladd Air Force Base, Alaska.*

Previous investigators have reported significantly higher basal metabolism in Eskimos than in Whites. This paper reports the results of 340 BMR tests in 73 healthy Eskimos from 4 different localities in Alaska. These studies include environmental observations, body surface measurements, food consumption and urinary nitrogen elimination. In agreement with previous workers the BMR of Eskimos in their native habitat was significantly higher than in Whites. In some cases a reduction of 20% in the BMR was observed when a subject was tested repeatedly while living on the same diet, although the BMR was still higher than in the White controls. The BMR was highest in the groups subsisting on a high protein diet, with urinary elimination as high as 3 gm/hour, varying with the different levels of protein intake. In a group of 14 Eskimos living on their native food, the average BMR was 8% higher than the DuBois standard. When given the white man's diet the average BMR was reduced to −8%, identical to a group of Whites living under identical conditions. Simultaneously the urinary nitrogen elimination was reduced to the same levels as observed in the White controls. When these Eskimos returned to their normal diets a rise in the BMR was observed. From these studies it may be concluded that there is no racial difference between the Eskimos and Whites in the basal heat production, and that the specific dynamic action of protein is an important factor in the high basal metabolism generally observed in the Eskimo examined in his normal native habitat.

Hypervitaminosis A in the dog. KAARE RODAHL (introduced by A. H. LAWTON). *Inst. of Physiology, Oslo Univ., Norway, and Arctic Aeromed. Lab., Ladd Air Force Base, Alaska.*

Toxic effect of polar bear liver has been reported in man and dogs in Arctic regions. In experiments on rats this was found to be due to the high vitamin A content of the bear liver (*J. Nutrition* 41: 3, 1950). An experiment in dogs is presented as an extension of comprehensive studies on hypervitaminosis A in other animals (*Norsk Polarinst. Skrifter* 95, 1950). The use of dogs greatly facilitates the study of the gradual changes which occur in hypervitaminosis A. An average of 387 IU vitamin A/gm body weight was given daily for 57 days. Following the first single dose weakness and drowsiness were observed within 1 hour and sickness 1 hour later. After 3 hours the dog had recovered from these symptoms of acute intoxication. Continued doses of vitamin A caused symptoms of chronic intoxication from the 5th day: anorexia, emaciation, weakness, pains in the limbs, alopecia, diarrhea, vomiting, bleeding and swelling of the gingiva, nose bleeding, and general muscular atrophy. Blood examination at intervals revealed gradual reduction of ascorbic acid and increase of vitamin A levels. Hemoconcentration, proteinuria and hematuria were detected. Urinary vitamin A excretion was increased. The x-ray examination of the long bones and postmortem and histological findings in the internal organs, teeth and bones were identical to those observed in hypervitaminotic rats, mice, guinea pigs and rabbits. The vitamin A content of the liver was 90,000 IU vitamin A/gm. When fed to rats characteristic symptoms of hypervitaminosis A developed. The similarity between symptoms of hypervitaminosis A and scurvy is discussed.

Pressure velocity relationships in production of murmurs. SIMON RODBARD. *Cardiovascular Dept., Med. Research Inst., Michael Reese Hosp., Chicago, Ill.*

Murmurs and thrills often occur in the absence of gross anatomical changes in the heart and great vessels. Even in the presence of demonstrable lesions, many abnormal sounds remain unexplained. Studies on animals and artificial circulation models demonstrate that many of these sounds are due to vibrations produced as a result of pressure-velocity relationships of the flowing blood; the vessel wall or valve actually being set into vibration. When a high velocity is imparted to the blood, the lateral pressure falls markedly (Bernoulli). This phenomenon is best observed at the site of a partial stenosis. The flutter effect may be seen to be due to the high velocity through the stenosed orifice which causes the lateral pressure at this site to fall far below atmospheric. In consequence the valvular walls tend to be pushed in. Stenosis thus becomes more severe, further increasing the velocity and lateral pressure falls still more. This process proceeds until the orifice is completely closed. Velocity now becomes zero, and the entire *vis a tergo* operates as lateral pressure; the walls of the orifice are thereby forced open. As flow recommences, velocity increases, lateral pressure falls, the cycle is repeated and a flutter effect is produced. Model experiments show that factors affecting the frequency of flutter include initial orifice area, head of pressure, elasticity of the vessel, and resistance beyond the stenosis. In aortic stenosis or coarctation of the aorta, the frequency of the anacrotic vibration is found to be a function of the degree of stenosis. Forces which produce these murmurs may also participate in the progression of stenotic lesions.

Nature of humoral mediator of pressor response to increased intracranial pressure. S. RODBARD, M. REYES* AND H. SAIKI.* *Cardiovascular Dept., Med. Research Inst., Michael Reese Hosp., Chicago, Ill.*

The lag of 6–10 seconds from the onset of increased intracranial pressure until the occurrence of the pressor response has been shown to be related to the circulation time. This suggests the intracranial compression causes a pressor material to enter the venules and, after being carried to the arterioles, its effect becomes manifest. We have utilized various blocking agents in an attempt to identify the pressor material. In the chick, the pressor response is blocked by previous administration of benzodioxane or Dibenamine; it is unaffected by tetraethylammonium chloride. The same pattern of response to these agents is obtained with intravenous nor-adrenalin. The results therefore suggest that at the onset of intracranial compression, neurogenic pathways to the periphery are stimulated, with the release of graded amounts of a nor-adrenalin-like compound into the blood stream. The implications of these findings in terms of blood pressure regulation are discussed.

Extraction and fractionation of urinary corticoids. LOUISE P. ROMANOFF,* ROSALIE S. WOLF* AND GREGORY PINCUS. *Worcester Fndn. for Experimental Biology, Shrewsbury, Mass.*

Two 24-hour urine samples are each incubated immediately after collections with beef spleen glucuronidase for 24 hours and then extracted continuously with CH_2Cl_2. The combined residues are then chromatographed on silica gel as previously described by us (*Federation Proc.* 9: 101, 1950). The benzene-ether fractions containing the less polar steroids (DOC-like) are combined and further fractionated on paper strips using the benzene formamide system (BURTON, ZAFFARONI AND KEUTMANN. *J. Biol. Chem.* 188: 763, 1951). The ether-ethyl acetate fractions containing such steroids as Kendall's compounds E and F are partitioned on paper strips using the propylene glycol-toluene system. The most polar steroids (steroid glycols) contained in the acetone and methanol eluates are combined and chromatographed on paper using a variation of the benzene-formamide system. The presence and characteristics of the steroids extracted are determined on the strips using such color reagent tests as blue tetrazolium chloride, dinitrophenylhydrazine, osmic acid, metadinitrobenzene, I_2 and $KMnO_4$. After elution of the steroid from the strips its UV adsorption in MeOH and its absorption maxima in H_2SO_4 are determined. If necessary for further identification, steroid acetates are made and partitioned using the benzene formamide system. Approximately 20 steroids have been regularly present in the human urines so far studied. These include steroids identified as Kendall's 17-hydroxycorticosterone, 11-dehydro 17-hydroxycorticosterone, allopregnane 3-β,11β,17α, 21 tetrol 20-one, allopregnane 3β,17α,21-triol-11, 20-dione, allopregnane 17α,21-diol-3,11,20-trione, allopregnane 3β,17α,21-triol-20-one and several steroid glycols. A number of steroids containing 3 and 4 oxygen atoms and having quite characteristic reactivities are present. These patterns of urinary corticosteroid excretion will be described in detail.

Alcoholic drive in rats treated with propyl thiouracil. B. ROSENBERG* AND M. X. ZARROW. *Dept. of Biological Sciences, Purdue Univ., Lafayette, Ind.*

An attempt was made to produce a state of alcoholism in the rat by interference with the thyroid gland. Adult male and female rats were used in this study and given a choice of water or 10% alcohol. A control period of 20 to 30 days preceded the addition of the goitrogen to the diet. After the normal drinking pattern had been established the rats were given a diet containing propyl thiouracil at concentrations of 0.2, 0.4 and 0.8%. In all instances the highest concentration of propyl thiouracil produced an alcoholic drive within 24 hours after the addition of the goitrogen to the diet. Lower concentrations produced a similar effect but required a slightly longer time. Several of the animals that failed to become alcoholic on the 0.2% goitrogen, did so when the thiouracil concentration was increased. The average ratio of water to alcohol consumption was 2.71 for the control group; 0.53 for the 0.2% propyl thiouracil group; 0.31 for the 0.4% group and 0.28 for the 0.8% group. This would indicate an increased alcohol consumption with increasing amounts of the goitrogen. In an attempt to determine whether this phenomenon was due to the antithyroidal action of the propyl thiouracil, a group of 'alcoholic' rats on a diet of 0.4% thiouracil were injected daily with 20 to 40 μg. of thyroxine. The high alcohol intake was not affected by this treatment. Furthermore thyroidectomy failed to make the rats alcoholic. Upon cessation of thiouracil treatment all rats lost their alcoholic drive and reverted to a normal drinking pattern. (Aided by a grant from the National Research Council, Committee on Problems of Alcohol.)

Latency of neural components in round window response to pure tones. WALTER A. ROSENBLITH AND MARK R. ROSENZWEIG (introduced by S. S. STEVENS). *Massachusetts Inst. of Technology, Cambridge, and Univ. of California, Berkeley.*

When low frequency tones are presented to the ear of anesthetized cats neural and microphonic components can be identified in the response recorded by wire electrodes from the round window of the cochlea. The action potentials ('neurals') behave like those recorded in response to acoustic clicks: they are sensitive to prolonged stimulation—exhibiting both equilibration and fatigue—and to temperature change. The position of these neurals in the cycle of microphonic potentials varies in a consistent manner with stimulus frequency and intensity. The following straight line equation describes the position of the neural in a given cycle (data from 7 animals): $\tau = aT + \tau_0$. T is the period of the stimulus tone and τ is the time interval separating the peak of the neural from the 0-degree crossing of the time axis in a sinusoid; τ_0 is the intercept of the straight line with the τ-axis, its value increases with decreasing stimulus intensity; a is the slope constant of the straight line, its value is about $\frac{1}{3}$ and apparently independent of stimulus intensity. This equation can be interpreted as follows: for equal-intensity tones of frequency less than 600 cps the latency of the neurals is constant when measured from the 180-degree crossing of the time axis of the sinusoid. (Research carried out at the Psycho-Acoustic Laboratory under contract N5ori-76 between Harvard Univ. and the ONR, U. S. Navy.)

Hypercholatemia as a cause of hypercholesteremic states. RAY H. ROSENMAN,* MEYER FRIEDMAN AND SANFORD BYERS. *Harold Brunn Inst., Mount Zion Hosp., San Francisco, Calif.*

Studies from this laboratory have indicated that experimental procedures as diverse as *1*) ligation of the bile duct, *2*) production of nephrosis, *3*) injection of 'Triton WR 1339' and *4*) induction of hypothyroidism led to accumulation of plasma cholate in the rat; this excess cholate was responsible for the hypercholesteremia also observed after the above procedures. Excess cholic acid is primary, and an adequate cause for the hypercholesteremia which is secondary to and preceded by hypercholatemia. The above experimental observations led to an investigation of a possible similar relationship between cholic acid and cholesterol in man. A group of 20 normocholesteremic subjects and 33 hypercholesteremic patients were studied. A highly significant correlation ($r = 0.834$) was found between the cholic acid and cholesterol levels in the sera from this group. The greater the concentration of cholate, the greater also the serum level of cholesterol. This correlation was independent of the particular syndrome associated with the hypercholesteremia. These findings suggest that clinical hypercholesteremia may be secondary to hypercholatemia.

Resistance to airflow during the respiratory cycle. B. B. ROSS (introduced by A. B. OTIS). *Dept. of Physiology and Vital Economics, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Changes in resistance to airflow in the tracheobronchial tree may be brought about by changes in the geometry of the lung and changes in the magnitude of turbulent airflow. In terms of pressure required to produce a given flow velocity, resistance is described by the equation $P/\frac{dV}{dt} = K - K'\frac{dV}{dt}$ where K is influenced by the effective length and diameter of the tracheo-bronchial tree and K' by the effective angle of divergence of the branches of the tree. P is obtained from a catheter inserted through a tracheal cannula into

a small bronchus of an anesthetized dog. This method is believed to give a more valid estimate of alveolar pressure than either the interruption method (OTIS AND PROCTOR, *Am. J. Physiol.* 1948) or the intrapleural pressure method (NEERGAARD AND WIRZ, *Ztschr. Klin. Med.* 1927). Resistance values, obtained without respect to time, plotted against flow velocity show turbulent resistance to be a linear function of velocity. By comparing resistance values obtained at specific times during the respiratory cycle with the above, the effect of changes in the configuration of the tracheobronchial tree may be evaluated. Any deviations would indicate a change in the constants K and K' previously described. Such a comparison reveals no significant change in resistance attributable to changes in the geometry of the lung tree during a respiratory cycle.

Physiologic effects of anterolateral chordotomy in man. GRACE M. ROTH, DONALD A. JOHNSON AND WINCHELL MCK. CRAIG.* *Mayo Fndn., Univ. of Minnesota and Mayo Clinic, Rochester.*

Chordotomy or the partial division of the anterolateral column of the spinal cord of man was first carried out by Spiller and Martin in 1911 for the treatment of intractable pain. The somatic arrangement of the fibers in the spinothalamic tract has been studied extensively and it is generally accepted that the more peripheral fibers represent the lower or caudal portion of the body while the medial fibers represent the upper portion although there is considerable overlapping of adjacent segments in the tract. However, little is known as to whether the autonomic fibers are distributed diffusely or segmentally in the anterolateral columns. In order to gain some information in regard to the autonomic nervous system, skin temperatures of the fingers and toes, and sweating tests were carried out on 7 patients after anterolateral chordotomy. Four of these patients had unilateral chordotomy and 3 bilateral. In 4 of the 7 patients the depth of surgical division was 5 mm., in 2 others it was 6 mm. (1 with unilateral chordotomy and the other with bilateral). In the 7th patient who had bilateral chordotomy it was 7 mm. The sweating pattern, changes in skin temperature and loss of pain perception have been correlated with the level and depth of the anterolateral chordotomy. The irregular distribution of the sweating pattern may be due to peripheral overlapping rather than to an absence of central segmentation. Severe orthostatic hypotension occurred in only 1 patient after bilateral chordotomy.

Effects of dinitrophenol (DNP) on endogenous respiration and fermentation of yeast. ASER ROTHSTEIN AND HARRY BERKE.* *Div. of Pharmacology, Dept. of Radiation Biology, Univ. of Rochester, School of Medicine and Dentistry, Rochester, N. Y.*

Although starved yeast does not normally ferment its stores of carbohydrate, in the presence of appropriate concentrations of dinitrophenol, an alcoholic endogenous fermentation takes place. The DNP-induced endogenous fermentation seems to involve different metabolic pathways than does the DNP-stimulated endogenous respiration. Firstly, different concentrations of DNP are required to produce the two effects. Secondly, the two effects can be differentiated by uranyl ion. The DNP-induced endogenous fermentation is relatively insensitive to uranyl ion whereas the DNP-stimulated endogenous respiration is completely inhibited. The DNP-stimulated endogenous respiration resembles eogenous respiration of glucose, rather than the normal endogenous respiration. Both the exogenous respiration and DNP-induced endogenous respiration are inhibited by CO and also by uranyl ion, whereas the normal endogenous respiration is insensitive to both CO and uranyl ion.

Separation of ketosteroids in a 24-hour urine sample. BETTY L. RUBIN* AND HARRIS ROSENKRANTZ* (introduced by O. M. HECHTER). *Worcester Fndn. for Exptl. Biology, Shrewsbury, Mass.*

A routine procedure has been devised for separating the ketonic, nondigitonin-precipitable steroids in a 24-hour urine sample by silica gel chromatography. A ratio of silica gel to 17-ketosteroids (17KS) of 1000:1 is used and the 17-KS content of each eluted fraction is followed by the Zimmerman reaction. Eluent mixtures progress in polarity from 25% benzene in petroleum ether through benzene to various ether-benzene mixtures. A total of 145-40 ml. fractions are collected. The columns are eluted over several working days. Trial runs with mixtures of pure compounds have shown that $\Delta^{2 \text{ or } 3}$-androstenone-17, androsterone, etiocholanol-3α-one-17, and 11-hydroxyandrosterone can be satisfactorily separated. From urinary extracts, 9 major components were obtained. These materials have been studied by infrared absorption analysis. Results will be presented on urines from normal and schizophrenic young men. (This work has been aided by a cooperative project with the National Institute of Mental Health, U. S. Public Health Service, Federal Security Agency.)

Effects of oxygen at high pressure on central nervous system axons. DONALD O. RUDIN AND GEORGE EISENMAN (introduced by HENRY K. BEECHER). *Anesthesia Lab. of Harvard Med. School at Massachusetts General Hosp., Boston.*

Adequately oxygenated axons of cat's spinal

tracts (RUDIN AND EISENMAN, *Science* 114: 300, 1951) and peripheral nerve equilibrated with Kreb's solution *in vitro* were subjected to 8 atm. oxygen pressure and studied oscillographically for excitability changes. Carbon dioxide tension was kept constant at 0.047 atm., and the extracellular space was restricted by holding the tissues in the gas phase of the chamber during exposure to high pressure oxygen. The latter procedure enhances changes in action potential properties induced by small shifts in ion number between intracellular and extracellular compartments, since changes in extracellular concentrations will be magnified thereby. Unlike continuous washing in an 'unlimited' extracellular volume this technique can imitate the role, *in vivo*, of the blood-brain barrier if one considers that it may restrict transport of certain ions into or away from the extracellular space. In the gas phase glucose supply is not limited during the first hour. Excitability controls under these conditions in 1 atm. oxygen with or without additional 7 atm. nitrogen pressure show relatively slow drifts. Under 8 atm. oxygen CNS axons (with 'L fraction membrane potential') show excitability increase beginning in 2 minutes, reaching peak in 25 followed by inexcitability in 50. This is irreversible unless tissue is washed in Kreb's solution. Peripheral nerve requires twice as long for similar changes but they are partly reversible. The initial character and onset time, at least, of ionic shifts postulated as directly responsible for the phenomenon cannot be due to extracellular volume restriction. Further they are of a type and have an onset time which could produce the CNS effects of oxygen poisoning in intact animals. Either membrane depolarization (increased extracellular potassium) or decreased impedance (calcium-binding), which produce the excitability changes here observed, is a likely net effect of oxygen at high pressure.

Studies of left ventricular filling. ROBERT F. RUSHMER, DEAN CRYSTAL,* CLYDE WAGNER,* JOHN HENDRON* AND RICHARD ELLIS.* *Dept. of Physiology and Biophysics, Univ. of Washington School of Medicine, Seattle.*

To initiate an investigation of reflexes influencing ventricular filling and ejection, ventricular size was recorded in unanesthetized animals by a new cinefluorographic method, not involving rapid injection of radio-opaque substances. Small metal markers were implanted surgically on opposite sides of the left ventricular cavity. Changes in ventricular diameter during successive cardiac cycles were determined by measuring the distance between these markers on cinefluorographic films (15 frames/sec.). Plotted against time, the changes in ventricular diameter during filling and emptying resembled cardiometer records described by others. About 2 weeks postoperatively, most of the animals developed bradycardia and sinus arrhythmia which gradually diminished during the next few weeks. The pattern of filling and emptying altered from beat to beat during arrhythmia. The increase in ventricular diameter during diastole was related to the duration of the diastolic interval. However, ventricular expansion during early diastole was similarly correlated with the duration of diastole so depression of pacemaker activity and increased ventricular distensibility may be caused by a common factor. To supplement direct linear measurements of ventricular diameter, a method will be described for the measurement of impedance across the left ventricular cavity utilizing the metal markers as electrodes. (This investigation was supported in part by a research grant from the National Heart Institute of the National Institutes of Health, Public Health Service.)

Alleged deterioration of intestinal transplants with time. RAYMOND A. RUSSELL* AND E. S. NASSET. *Dept. of Physiology and Vital Economics, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

The intestinal secretion of a dog's subcutaneous jejunal transplant, open at both ends and with intact mesenteric pedicle, was measured at intervals for a period of 51 months. Secretion was elicited in the fasting and fed animal by the insertion of smooth catheters. A progressive decline in volume output of this fistula occurred. Changes in the abdominal wall surrounding the transplant, rather than in the gut itself, are believed to have facilitated the retention and reabsorption of secreted fluid. Since the enzymes are not thus reabsorbed, the enzyme output provides a more reliable criterion of secretory activity. By an improved method of enzyme collection it was shown that no change in enzyme output (invertase) accompanied this decline in volume output. Previous workers have overlooked the fact that a major part of the enzyme may remain within the fistula in a semi-solid state when the catheters are withdrawn. The fraction thus lost increases with decreasing volume output, another fact which has led others to the erroneous conclusion that the enzyme output also declines. Histological examination of the intestine and pedicle 51 months after establishment of the fistula revealed both to be entirely normal. The gut wall showed many normal ganglion cells, and the pedicle displayed 22 nerve trunks in apparently functional condition. No evidence was adduced for the spontaneous and eventually complete denervation of such fistulae alleged by Florey, Wright and Jennings (*Physiol. Rev.* 21: 36. 1941).

Studies of F_1 generation hybrid mice from an irradiated parent. JOHN H. RUST, ANN M. BUDY AND ROBERT D. BOCHE (introduced by FRANKLIN C. MCLEAN). *UT-AEC Agricultural Research Program, Oak Ridge, Tenn., and Univ. of Chicago USAF Radiation Lab., Chicago, Ill.*

Experiments in which a single dose of 200 r of x-radiation delivered to C_{57} male and female mice were done to ascertain whether this exposure would produce an effect on life-span of offspring or litter size when the irradiated animals were mated with untreated dba mice. Matings of the same pairs were made 56 days prior to (controls), immediately after exposure, 120 and 180 days following radiation. At 210 days, brother-sister matings of the parents were made. All parents were sacrificed at the termination of the breeding phase of this study. Irradiation of male and female mice has produced no significant reduction in litter size from matings following irradiation in a series of 45 pairs. There were no litters born of irradiated C_{57} females after 120 days or subsequently following irradiation. About 2% of the irradiated C_{57} males were able to produce offspring at 120 and 180 days following irradiation. Life-span data on offspring of an irradiated parent, observed thus far, show no significant difference between the control and the postirradiated litters with respect to weight, general appearance and survival. The ovaries of irradiated females were without follicles thus accounting for the fact that there were no pregnancies among them when mated at 120 days and later. In nonirradiated females active oogenesis was apparent and all ovaries had recently formed corpora lutea. Spermatogenesis in the irradiated males was not as uniform among the tubules as it was in the nonirradiated and frequently the epididymides were not filled with sperm.

Effect of insulin on phosphate transport and glucose metabolism. JACOB SACKS AND F. MAROTT SINEX.* *Biology and Med. Depts., Brookhaven Natl. Lab., Upton, N. Y.*

A comparison has been made of the effect of insulin on phosphate turnover, glucose oxidation, and glycogen deposition in the isolated rat diaphragm incubated in a medium containing phosphate labeled with P^{32} and glucose uniformly labeled with C^{14}. Insulin increased the turnover rates of the acid-labile $\dot{P}$ of ATP, phosphocreatine, and hexosemonophosphate, each to about the same extent, i.e. 50% in the first hour of incubation. Insulin increased the oxidation of glucose from the medium by this same factor. On a molar basis, the increased phosphate turnover produced by insulin was 5 times as great as the increase in glucose from the medium oxidized during the first hour of incubation, and twice as great as the increase in the deposition of C^{14} glucose as glycogen. Less than one-third of the glycogen deposited under the influence of insulin appears to have been derived from the glucose of the medium. (Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

Stimulation by corticotropin of formation of corticoids by isolated rat adrenals. MURRAY SAFFRAN, BERNARD GRAD AND MARGARET J. BAYLISS (introduced by R. A. CLEGHORN). *Allan Memorial Inst. of Psychiatry, McGill Univ., Montreal, Canada.*

Bisected rat adrenal glands, incubated for 2 hours in Krebs-Ringer-phosphate-glucose medium in an atmosphere of oxygen, produce corticoids (0–11 γ/100 mg. adrenals), as estimated by the Speirs-Meyer bioassay (*Endocrinology* 48: 316, 1951) and by the absorption of light at 240 mμ by chloroform-soluble substances. The formation of corticoids is increased up to 45 γ/100 mg. adrenals by the addition of 10–1000 γ corticotropin (ACTHAR, Armour) to the medium. The amount of the increase is proportional to the amount of corticotropin added within this range. The effect of corticotropin is absent at 2°. (This investigation was supported by funds from the Dominion-Provincial Mental Health and Public Health Grants and from the Defence Research Board.)

Fluid balance and electrolyte dialysis in relation to plasma substitutes. STELIOS C. SAMARAS AND NICHOLAS DIETZ, JR. (introduced by FRANKLIN C. MCLEAN). *Dept. of Biochemistry, Creighton Univ. Med. School, Omaha, Nebr.*

The animal organism normally provides fluid and electrolyte balance necessary for proper tissue and cellular functions. However after hemorrhage, shock etc., blood, plasma or substitute transfusions may be necessary to restore a suitable physicochemical milieu for continuation of vital functions. As knowledge of the action of substitutes is incomplete, 374 dialysis experiments and 80 controls were performed. Forty ml. portions of substitute in 0.85% NaCl were placed inside, and 40 ml. portions of water outside, cellophane dialyzing tubes; 0.85% NaCl without plasma substitute was used in the controls. Separate humidity-controlled groups of experiments were maintained at 30°–31°C. for 10, 30, 60 minutes, and 2, 6, 12, 24 hours. After the respective time intervals, the fluid volumes inside and outside the membranes were measured and their chloride concentrations determined. Substitutes used were acacia, pectin, 'graplasmoid-Life,' polyvinylpyrrolidone and gelatin. Average data, based on 10 or more experiments for each time interval, indicated that fluid volume inside the membrane was practically unaffected up to 6 hours in all experi-

ments. In the 12 to 24 hour saline controls, inside fluid volume decreased slightly. Addition of acacia, gelatin, graplasmoid or polyvinylpyrrolidone slightly increased inside fluid volume, but pectin (0.75%) had no influence. Experimental and control chloride concentrations ran generally parallel, the inside NaCl decreasing to 0.39%–0.56% in 24 hours. As the substitutes did not significantly modify chloride dialysis, we believe that the volume increases were probably due to their water holding and binding capacities.

Effect of thyroxin pretreatment on decarboxylation of dihydroxyphenylalanine (dopa) and production of sustained hypertension. A. E. Samiy (introduced by William G. Clark). *Depts. of Zoology and Physiological Chemistry, Univ. of California, Los Angeles.*

Goodall (*Acta Chem. Scandinav.* 4: 550, 1951) demonstrated in addition to hydroxytyramine, dihydroxyphenylalanine (dopa) in the adrenals of thyroidectomized but not of normal sheep. This suggested to us the possible importance of the thyroid in dopa decarboxylation. Since hydroxytyramine, the decarboxylation product of dopa, is a pressor agent, blood pressure changes were taken as an indication of decarboxylation. Rats were injected subcutaneously with 6 mg/kg. *l*-thyroxin for a week, then with *dl*-dopa, 80 mg/kg. for a subsequent week. Dopa controls were normotensive whereas hyperthyroid rats developed 190 mm. Hg systolic B.P. after a week of dopa administration. Thyroxin-treated rabbits given dopa for a week developed a B.P. of 162/100 as compared with 100/75 in the controls. Doses of thyroxin less than 1 mg/kg. were ineffective. Thyroxin-treated rabbits had increased heart and kidney weights but not adrenals, but in thyroxin treatment + dopa, the adrenals became hypertrophied. Thyroxin treatment alone raised the systolic B.P. in both species, but to a less extent than when dopa also was given, and it returned to normal sooner. Pressor assays in cats showed much greater activity (3×) of urine from thyroxin-treated rabbits given dopa than those given dopa only. Thyroxin-treated male rats developed a much greater hypertension than females. Holtz & Credner (*Arch. exper. Path.* 200: 365, 1942) showed a pharmacological tests of urine, that rabbits decarboxylated dopa to hydroxytyramine. In the present work with rabbits, this was confirmed by paper chromatography. A total of 300 rats and 25 rabbits was used. (Supported by grants from Life Insurance Medical Research Fund and ONR.)

Some effects of short chain fatty acids on phosphate and carbohydrate metabolism of yeast. Frederick E. Samson,* Arnold M. Katz,* Daniel L. Harris* and J. Z. Hearon. *Dept. of Physiology, Univ. of Chicago, Chicago, Ill.*

The rapid phosphate uptake (measured chemically and by P^{32}) which occurs normally when yeast cells are fermenting glucose is completely inhibited if acetate or another fatty acid is present in the medium. The fermentation of glucose, measured as anaerobic CO_2 production, is inhibited by fatty acids, but to a lesser extent. The respiration of ethanol and glucose is inhibited. The pH studies indicate that the undissociated fatty acid is the active agent in these inhibitions. Studies with cell free extract show that the inhibition of fermentation is not a result of lowered intracellular pH or of membrane phenomena. There is a direct relationship between hydrocarbon chain length and extent of inhibition. The fermentations of glycogen, hexose-diphosphate, and 3-phosphoglyceric acid by cell free extract are also inhibited. The decarboxylation of pyruvate is enhanced. Tricarboxylic acid cycle intermediates, other than acetate, fail to show this inhibitory action. The data indicate that fatty acids may inhibit more than one enzyme system. These inhibitory actions of fatty acids and the well known dependence of fat metabolism on carbohydrates suggest that interplay between carbohydrates and fatty acids has an important role in the regulation of cellular metabolism. (Supported in part by a U. S. Public Health Fellowship and the Wallace C. and Clara Abbott Memorial Fund.)

Membrane processes in excitation-contraction (E-C) coupling. Alexander Sandow and Arthur J. Kahn.* *Dept. of Biology, Washington Square College of Arts and Science, New York Univ., New York City.*

The latent period of muscular contraction consists of an initial mechanically quiescent interval, L_R, followed by the latency relaxation, LR. The LR signalizes activation of the contractile system. The L_R interval includes 2 kinetically separable E-C coupling processes localizable in the muscle fiber membrane: the action potential spike, and the spike-activation link. Under massive stimulation all excitation and contraction events are normal, except that, due to simultaneity of response of all points along each fiber, propagated disturbances are absent. Hence, massive excitation involves a normal ('massive') spike potential process, but includes no local circuit currents. Therefore the spike potential, not local currents, initiates E-C coupling. Muscles treated with extra K or reduced Na develop greatly decreased spike potentials, yet show normal or even potentiated twitch tensions; hence the spike acts as a trigger with a large safety-factor. The other membrane reaction is indicated by enhancements of both LR and twitch tension due to short-time action of extra K. This action is shown, by a diffusion analysis of the passage of the K into the muscle, to be exerted directly only at the fiber membrane.

Thus, in addition to the spike, which is depressed by any extra K, another membrane process, the spike-activation link, is inferred which is enhanced by extra K, is triggered by the spike, and is propagated inward to determine activation of the contractile mechanism. (Aided by Contract NR113-300 with the Office of Naval Research.)

Latent addition in inexcitable muscle. ALEXANDER SANDOW AND ARTHUR J. KAHN. *Dept. of Biology, Washington Square College of Arts and Science, New York Univ., New York City.*

Frog sartorii made inexcitable by treatment with extra K, procaine, eserine, or complete Na-lack (choline or sucrose replacement), and stimulated by intense, massive shocks, produce moderately strong, twitch-like contractures. (*Federation Proc.* 6: 195, 1947.) Latent addition in the initiation of such responses has now been studied using the dual-shock technique. Square-wave shocks (100 μsec.) are used, the first 90% of threshold. The summated effect of each 2-shock sequence was detected mechanically by the piezoelectric technique as a threshold response of the most sensitive fiber (or fibers) of the curarized muscle. Normal curarized muscles exhibit a decay of the excitatory disturbance, due to the initial subthreshold shock, indicating, in some cases, evidence of a local response, and showing an apparent time-constant of the order of 25–50 μsec. Muscles made inexcitable by the previously mentioned treatments, all showed decay curves similar to the normal, but the time-constant was increased about tenfold. Strength-duration curves also prove low 'excitability' of treated muscles, since as compared to normal curves, they are each much shifted upward and to the right. Treatment of muscles with varying degrees of Na-lack resulted in progressive effects, e.g. the time constant of the excitatory disturbance averaged about 125 μsec. for 50%, 300 μsec. for 75%, and 370 μsec. for 100% lack. The results with completely inexcitable muscle indicate that electric shocks set up a membrane state similar to that of normal, but much slower in time characteristic, and which can lead to a modified activation of the contactile system in some respects similar to that of normal excitation-contraction coupling. (Aided under Contract NR113-300 with the Office of Naval Research.)

Total body sodium in hypertension in rat. LEO A. SAPIRSTEIN AND ROBERT GREENE.* *Dept. of Physiology, Ohio State Univ., Columbus.*

The total sodium content of 12 normal and 9 renally hypertensive rats was determined by wet ashing of the entire body. Potassium and nitrogen contents were determined on the same digests. The sodium content of the normal rat was 1.30 mg. Na/gm. The nitrogen content was 29 mg/gm. and potassium was 2.5 mg/gm. In hypertension sodium was elevated to 1.5–2.0 mg/gm. while potassium and nitrogen remain within normal limits or were slightly elevated. Plasma sodium remained normal in the hypertensive animals. Since nitrogen and potassium are located almost entirely in the intracellular compartment, the increase in sodium cannot be explained on the basis of an expansion of the extracellular compartment unless an extreme intracellular dehydration, inconsistent with the normal plasma sodium level is postulated. The data must therefore be interpreted to indicate increased penetration of the intracellular compartment by sodium. The fact that potassium values are not lowered indicates that the increase in intracellular sodium occurs at the expense of some intracellular cation other than potassium (perhaps magnesium), or that the intracellular sodium is in an osmotically inactive state. (Supported by a grant from the American Heart Association.)

Estimated changes in pulmonary blood volume accompanying systemic vasoconstriction and vasodilation using a gravimetric method for continuously recording the changes. STANLEY J. SARNOFF AND ERIK BERGLAND.* *Dept. of Physiology, Harvard School of Public Health, Boston, Mass.*

After splitting the sternum and retracting the sides of the chest laterally, both lungs were suspended by affixing one or more ligatures to the ventral edge of each lobe and bringing these ligatures to a central point above the chest, where they were attached to a 200 cc. Erlenmeyer flask. This was, in turn, suspended from a sensitive lever. The other end of the lever was attached by a string to a water-filled phosphor-bronze bellows in which pressure was recorded. Calibration was done by adding known volumes of water to the flask from which the lungs were suspended and was found to be linear. Pressures in the vena cava, pulmonary artery, left auricle, and femoral artery were also recorded by electromanometers. Systemic vasoconstriction, induced by the injection of thrombin and fibrinogen into the cisterna magna, was accompanied by left auricle and pulmonary artery hypertension and a striking increase in pulmonary weight (blood volume). Systemic vasodilation with Ro 2-2222 completely reversed these changes. By relating left auricle pressure changes with pulmonary weight (blood volume) changes, an estimation of the *in vivo* pressure-volume curve of the pulmonary vascular bed was obtained. This agreed in general with the curves previously obtained in the isolated lung.

Effects of denervation on renal function in the dog. O. W. SARTORIUS AND H. BURLINGTON.* *Dept. of Physiology, State Univ. of New York Med. Center, Syracuse.*

The alterations in renal function produced by acute renal denervation has been investigated in the anesthetized and unanesthetized dog and in crossed perfusion experiments. In the initial type of experiment, healthy female mongrel dogs were anesthetized and the renal nerves of both kidneys carefully isolated. Silk thread was placed loosely about the nerve bundles and carried to the outside through a flank incision. By means of a midline bladder incision, ureteral catheters were inserted into the vagina and ureters and securely tied. It was thus possible on succeeding days to collect urine from each kidney and, upon the collection of satisfactory control periods, to acutely denervate either kidney in the anesthetized or unanesthetized dog by a single pull on the string. In the crossed perfusion type of experiment, the left kidney received its entire blood supply from the carotids of a donor dog, while its nerves remained intact for control purposes. With this preparation, it was thus possible to study the effects of denervation on renal plasma flow, glomerular filtration rate, and tubular reabsorptive activity at various filtration pressures. In all experiments on anesthetized dogs, acute denervation resulted in an increased excretion of water and salt with no significant alteration in glomerular filtration rate or renal plasma flow on the denervated side. With the establishment of a diuresis in unanesthetized dogs, acute denervation resulted in a decreased urine flow, again with no significant alteration in hemodynamics. Crossed perfusion experiments have confirmed and supplemented the above, thus suggesting a direct influence of the renal nerves upon the reabsorptive activity of the renal tubules.

Progesterone initially facilitates and later inhibits release of pituitary ovulating hormone in the rabbit. CHARLES H. SAWYER. *Depts. of Anatomy, Duke Univ. and University of California, Los Angeles, and Dept. of Investigative Medicine, Long Beach Veterans Administration Hosp., Long Beach.*

So much emphasis has been given the inhibitory effects of the steroid that the several reports of facilitation of ovulation by progesterone have commonly been overlooked. In the rabbit, a species which ordinarily ovulates only after neurogenic stimulation of the adenohypophysis at coitus, we have evidence that progesterone facilitates and subsequently inhibits release of pituitary ovulating hormone. Of 40 female rabbits, estrous or estrogen-primed, treated with 2 mg. progesterone subcutaneously and subjected within 1–4 hours to glass-rod stimulation of the vagina, 11 ovulated as compared with only 2/30 controls. Mating or intravenous copper acetate within 4 hours after injection of progesterone stimulated ovulation in all of 10 estrous rabbits (5 each). Ten anestrous animals failed to respond to progesterone and artificial stimulation by ovulating. A few estrogen-progesterone-treated females ovulated 'spontaneously.' The facilitation appears to depend on synergism of progesterone with estrogen. On the other hand, of 17 estrous or estrogen-primed rabbits treated with progesterone and permitted to mate 24 hours later, only 10 would mate and 8 of these failed to ovulate. None of 10 females receiving artificial vaginal stimulation under these conditions ovulated. Furthermore, only 9/17 rabbits injected with copper acetate 24 hours after progesterone revealed ruptured follicles. These results confirm the often-observed inhibitory effect of progesterone, but the present work emphasizes that preceding this phase there is a period of facilitation of the ovulatory response.

Effect of increased alveolar carbon dioxide tensions on various physiological functions. K. E. SCHAEFER, E. R. CORNISH AND S. E. STUNTZ (introduced by E. S. MENDELSON). *Physiology Branch, U. S. Naval Med. Research Lab., New London, Conn.*

Thirty-nine subjects were exposed for 15 minutes to 3.3, 5.4 and 7.5% CO_2 in air. Each CO_2 exposure was followed by a 15-minute period on air. Alveolar CO_2 and O_2 tensions were continuously recorded (partly with Rahnsampler and Cambridge unit, partly with infrared CO_2 analyzer). Compared with laboratory personnel, a group of instructors at the Escape Training Tank showed a significantly lower ventilatory response to 5.4 and 7.5% CO_2 in air and to 10.5% O_2 in N_2. After transition from CO_2 to air, a hypocapnia effect (drop in alveolar CO_2 below initial level) was noted in 8 subjects only. This effect was more apparent after 5.4% CO_2 than after 3.3 and 7.5% CO_2. A steady state of ventilation and alveolar CO_2 and O_2 tensions was reached under 7.5% CO_2 after 10 minutes. In the 10–15-minute periods on CO_2 and air measurements of central fatigue (inhibitory effects of light stimuli on alpha waves, 4 leads) and alertness (muscle potential output of the forehead) were made. Increased alveolar CO_2 tensions reduced significantly the inhibitory effect of light stimuli on alpha waves, and the increase in muscle potential output produced by light stimuli and opening of the eyes. Alertness as measured by the muscle potential output of the forehead (independent of light stimuli) did not show clear relationships with the alveolar CO_2 tensions. Compared with the preceding period on air, alertness was reduced under 3.5% and CO_2 increased under 5.4 and 7.5% CO_2. Activity of the adrenal medulla and adrenal cortex was stimulated by increased alveolar CO_2 tensions, as shown in the rise in blood sugar and the drop in absolute number of eosinophils durings the 10–15-minute period of exposure to 3.3, 5.4, and 7.5% CO_2.

Spread of electrical activity through the wall of the mammalian ventricle. ALLEN M. SCHER, RICHARD R. PATON AND ALLAN C. YOUNG (introduced by HENRY N. HARKINS). *Dept. of Physiology and Biophysics, Univ. of Washington School of Medicine, Seattle.*

Multipolar electrodes were constructed of fine (0.001″) tungsten wires staggered along a central shaft of 0.005″ tungsten. The recording ends of the wires were separated by 1 mm. or less. When these were inserted perpendicularly into the ventricular wall it was possible to trace the excitation through the entire thickness. Control ECG's showed no changes from insertion of these electrodes. A fine needle electrode in the auricle was led into a delayer amplifier which triggered the sweep of a dual channel oscilloscope. One channel was used to record a time reference from a needle electrode in the ventricle throughout an experiment. The other channel recorded from the multipolar electrodes which were inserted at many places in the ventricle. In early experiments, monopolar, bipolar and tripolar records were taken and it was found that the time of excitation of a point could be accurately gauged from a bipolar electrode. Excitation of the ventricular wall was always found to proceed from endocardium to epicardium and was free of discontinuities when time of excitation was plotted as a function of time even though different muscle bundles were pierced. Velocity of spread for different ventricular areas and different animals varied from 150–500 mm/sec. and was quite consistent for a given electrode placement. Electrodes in the septum gave bizarre patterns without orderly sequence of excitation. Papillary muscles were activated centripetally from their attachment at the ventricular wall toward the cavity. Evidence was obtained that an electrode may move slightly during the several heartbeats required to take records and further experiments will require multi-channel recording.

Disappearance rate of fluorescein conjugated plasma proteins from the circulation of hypoxic rats. ALFRED A. SCHILLER AND GLORYE N. WOOL.* *Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.*

Thirty six male albino rats were given fluorescein conjugated homologous plasma proteins (25 mg/100 gm.) intravenously. Alternate animals were placed singly into a low pressure chamber and brought to a simulated altitude of 18,000 feet for 3 hours. A few animals were also exposed to simulated altitudes of 28,000, 30,000 and 34,000 feet for a comparable period of time. The remaining group of animals were kept at ground level and served as controls. Blood samples from tail veins were obtained just prior to altitude exposure and again immediately afterwards and analyzed fluorophotometrically for the concentration of the fluorescein label. 'Half-life' constants of the labeled protein in the circulation for the 3-hour period in both experimental and control animals were calculated from the slopes of curves obtained from semilog plots of label concentration as a function of time. Since the disappearance rates at the several altitudes were not significantly different from each other, they were pooled and treated as a homogeneous series. The disappearance rates of the labeled proteins, expressed as 'half-life' constants were found to be 4.0 ± 0.9 hours for the control group and 3.9 ± 0.8 hours for the experimental group, the difference between groups being highly insignificant. These results suggest that in intact rats, grades of hypoxemia corresponding approximately to a pO_2 of 25 mm. Hg or an oxygen-hemoglobin saturation of 30% do not produce hemodynamic or permeability changes which significantly alter transcapillary passage of plasma proteins.

Effect of hemoglobin and adrenaline on urinary pH and electrolytes. JØRGEN U. SCHLEGEL, JOSEPH J. BETHEIL* AND A. LEONARD BLOCH.* *Depts. of Surgery and Biochemistry, Buswell Urological Research Lab., Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Using rabbits, the intravenous administration of homologous hemoglobin preparations (100 mg/kg.) resulted in a prolonged increase in blood pressure of 20 to 30 mm. Hg, a pronounced rise in the pH of the urine, and an increase in the excretion of sodium and water. Prior to the experiment all animals were starved overnight. The urine was collected through an indwelling catheter connected to a flow-type glass electrode assembly. The pH was continuously recorded for a period of 6–8 hours; after the first 2–3 hours the urine became acid and hemoglobin was then administered. The urine subsequently was free of casts. It was analysed for chloride, sulphate, phosphate, sodium and potassium. During the 5-day period following the administration of the hemoglobin preparation there was no oliguria and the blood urea nitrogen remained normal. Adrenaline, like hemoglobin, had the same effect on urine pH, water and electrolyte excretion. Clinical studies revealed the same effects of adrenaline in humans, except when a low plasma sodium was present. Regitine (Ciba), an adrenolytic substance, in dosages (4 mg/kg.) sufficient to counteract the blood pressure rise due to hemoglobin, was administered intravenously together with hemoglobin. All changes, which usually followed hemoglobin administration, were lacking. The concentration of hemoglobin in urine was greatly increased, numerous casts were present, and the blood urea nitrogen increased. It is suggested that

one of the etiological factors in hemoglobinuric nephrosis is a lack of normal adrenergic activity.

Kidney function in the kangaroo rat. BODIL SCHMIDT-NIELSEN. *Kettering Lab., Univ. of Cincinnati, Cincinnati, Ohio.*

It has been reported by the author that kangaroo rats are able to excrete urea by renal tubular excretion. In the present investigation the attempt has been made to confirm these results, using improved techniques for the clearance determinations. The animals are given a constant intravenous infusion through a tail vein and blood samples are taken from the foot. This technique has previously been worked out by Dr. E. Cotlove, National Heart Institute, Bethesda, Md., to whom the present author is indebted for his demonstration of the technique. Glomerular filtration rates were determined by measuring the inulin and, in some instances, also the mannitol clearance. High diuresis was induced in the animals by various diuretics such as mannitol, sodium chloride, salyrgan, etc. Water alone does not easily induce increased diuresis in kangaroo rats but results in water intoxication, probably because of a high concentration of antidiuretic hormone in the blood of these animals. As a result of the back diffusion of urea in the tubules it was not possible to demonstrate tubular excretion unless the diuresis was increased considerably. The finding of urea/inulin clearance ratios higher than unity will be discussed.

Oxygen dissociation of mammalian blood in relation to body size. KNUT SCHMIDT-NIELSEN AND BJÖRN GJÖNNES.* *Kettering Lab., Univ. of Cincinnati, Cincinnati, Ohio, and Dept. of Chemistry, Univ. of Oslo, Norway.*

The oxygen dissociation curves of mammalian blood, when measured at the same CO_2 pressure, seem related to the body size of the animal. When plotted in the coordinate system commonly used for such curves, the curves for small animals are found to the right of those for animals of large body size. Therefore, the oxygen of the blood will be unloaded at a higher pressure in small mammals than in larger mammals. This relation of the oxygen unloading tension to the body size may have significance in connection with the higher oxygen consumption of the tissues of small animals. The density of capillaries in small mammals is higher, but calculations show that, in addition, a greater oxygen pressure difference is required to establish a diffusion gradient sufficiently steep for an adequate supply of oxygen from the blood to the tissues. The following objections must be raised. The observation of a correlation between body size and the oxygen dissociation curve of mammalian blood was based largely on work of previous investigators. Too few determinations have been made near the extremes of body size of mammals. The state of these facts makes the attempt to interpret the observation as a factor in the oxygen supply to the tissues entirely hypothetical.

Direct visual examination of stereovectorelectrocardiograms. OTTO H. SCHMITT AND R. B. LEVINE.* *Depts. of Zoology and Physics, Univ. of Minnesota, Minneapolis.*

It is no longer technically difficult to record conventional electrocardiograms accurately and reproducibly, but it is still very difficult to interpret these patterns quickly and unambiguously. It would appear logical, therefore, that we should next attempt to shift some of the analytical burden from the operator to the instrumentation. In particular the spatial synthesis of vector components into a total vector pattern in space and time should be done instrumentally, not mentally. While vectorcardiograms and the composite vectorcardiograms which give some impression of spatial vector organization are a step in this direction, we believe that the small extra cost in instrumentation for full three dimensional stereovectorelectrocardiographic (SVEC) presentation is amply justified whenever more than conventional one-dimensional data against time are required. Accordingly we have developed and set up equipment which will routinely produce these SVEC patterns for direct viewing and for simultaneous photographic recording, and we are starting a series of measurements on a few hundred normal subjects and heart patients. These tests will allow us to establish norms and to test practically the image sizes, forms, and orientations which we have chosen theoretically as most advantageous. At the meetings we will show by polarized stereo projection some of these SVEC records and will discuss preliminary results and tentative standards.

Isolation of rat liver nuclei in glycerol. ROSE MARY SCHNEIDER (introduced by M. J. KOPAC). *Dept. of Biology, Washington Square College, New York Univ., New York City.*

Rat liver nuclei have been isolated in a neutralized hypertonic solution of the nonelectrolyte glycerol. This medium has been utilized for a study of conditions affecting optical clarity, which is a property of the living nucleus. One gm. of frozen liver, suspended in aqueous 70% glycerol neutralized with potassium hydroxide, is crushed, strained and homogenized at high speed at 2°C. The homogenate is then sedimented in a refrigerated centrifuge and the sediment washed twice with the glycerol solution. The final sediment consists of intact liver nuclei, dispersed and free of whole cells, red blood cells or extranuclear debris. Examination of the sediment is made under

immersion oil, using light-field, dark-field and phase microscopy. The nuclei isolated by this method are 'granular' in appearance. However, those isolated in glycerol containing metaphosphates, orthophosphates, oxalate, citrate or carbonate become progressively 'nongranular' or homogeneous (except for persisting nucleoli) as the concentration of the salt is increased. Ultimately the nuclei become hyaline and are often clustered as well. The phenomena of nuclear 'granularity,' hyalinity and clustering are being investigated with respect to both pH and concentration of effective salt. Consideration is also being given to identification of the particular cation bound by the anions employed.

Total tension and actively developed muscle twitch tension. GORDON M. SCHOEFFLE, A. S. GILSON, JR. AND WILLIAM W. SLEATOR, JR.* *Dept. of Physiology, Washington Univ. School of Medicine, Saint Louis, Mo.*

The ratio dl/dT was approximated by determining the change in length of an isotonically loaded turtle neck retractor muscle when a small weight was subtracted from the initial load. As initial load is increased dl/dT decreases but remains directly proportional to peak twitch deflection. This indicates that the actively developed twitch force which acts in parallel with elastic components of the stretched muscle is in itself a function only of the time and is not at all dependent on tension or length within wide limits. The time parameters of the 'slip' following sudden stretching of the resting muscles are entirely unaffected by a 15° drop in temperature while the time parameters of the twitch are markedly changed. Hence it seems most improbable that viscous forces are of any importance in determining more than the initial foot of the twitch configuration. These data were interpreted in relation to the previous finding that the value of modulus dT/dl is determined by the tension regardless of whether the muscle is resting or active. It is proposed that the muscle fiber may be analyzed in terms of series elements, each containing a weak and strong spring in series. The moduli of these springs are nonlinear functions of muscle length but bear a constant relation to each other in the isotonic system. In terms of this model the active force of the twitch is applied in parallel with the strong spring of each element.

Oxygen consumption during cleavage of single cells. P. F. SCHOLANDER, C. L. CLAFF* AND S. L. SVEINSSON.* *Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.*

A micro respirometer is described which is sensitive to 0.2 millionth mm.^{3} and has a stability of from 0–10 millionths mm.^{3}/hour. Principle: A minute respiration chamber is filled with a medium which communicates to the same medium on the outside through a fine capillary space. This space serves as a diffusion barrier, but permits free pressure transmission to the chamber from the outside. The respiration chamber contains besides the cell a minute reference diver cut from a plastic capillary filament. A bubble is generated onto the diver by lowering the pressure. The pressure is adjusted so that the bubble always just suspends the diver. The change in flotation pressure, multiplied by a factor, gives the oxygen consumption. The CO_2 is effectively held in solution by the large liquid ratio of the system. The oxygen consumption has been analyzed during the first 2–5 cell divisions in single eggs from 3 species of echinoderms and from *Urechis*. Cell division may proceed without measurable change in rate, or it may show acceleration or cycling correlated with the cell divisions. If cycling occurs the cytoplasmic cleavage is associated with an abrupt rise in the oxygen consumption. When started, cleavage can proceed without oxygen consumption. The cycling is strongly damped and often disappears after 2–3 divisions. Our runs on single eggs confirm results indicated by mass runs, but we are able to supply a more exact picture with respect to timing, curve shape, and variations in individual cells.

Dye injection curves in traumatic arterio-venous fistulae in man. GEORGE E. SCHREINER, NORBERT FREINKEL, JOHN W. ATHENS AND WILLIAM L. STONE III (introduced by ROBERT W. CLARKE). *Dept. of Surgical Physiology and Biophysics, Army Med. Service Grad. School, Walter Reed Army Med. Center, Washington, D. C.*

The accuracy of the cardiac output determination by the Hamilton modification of the Stewart technique is largely contingent upon ability to delineate the downslope. Little information is available on the effect of abnormal peripheral communications on the contour of the curve. Description of these effects and correlation with the site and size of the arterio-venous fistula might elucidate some of the factors affecting the contour of the arterial curve. T-1824 dye injection curves were determined by direct analysis of samples obtained at 2-second intervals from the femoral artery, in 7 patients with patent arterio-venous fistulas, 6 patients following operative removal of their fistulas and in 10 hospital controls. The patent fistulas included 2 popliteal, 3 femoral, 1 superficial femoral and 2 carotid arteries. In 3 patients it was possible manually to occlude the fistulas and by repeated injection to provide immediate demonstration of the differences in morphology of the curve with the fistula open and closed. Curves were analyzed in terms

of *1*) appearance time of the dye; *2*) up-slope time; *3*) disappearance time; *4*) clearance time (t); *5*) mean recirculation time; and *6*) interval to the beginning of manifest recirculation. The most divergent parameter was the mean recirculation time which averaged 19 seconds in the controls, 18.4 seconds on the postoperative cases and 11.4 seconds in the patent fistulas.

Central nervous system lesions and aggressive behavior in cats. L. SCHREINER,* A. KLING* AND R. GALAMBOS. *Army Med. Service Graduate School, Washington, D. C.*

Certain lesions, primarily those localized rostral to the midbrain, result in altered behavior outwardly manifested as aggression and savageness. Behavioral deficits of the opposite character (docility, mutism, and somnolence) are described following a variety of lesions, especially those of the caudal hypothalamus and midbrain. This report will describe, on a comparative basis, behavioral changes after placement of 'rage'-producing lesions as well as deficits in behavior following lesions in which aggression was not a postoperative finding. Postoperatively, noxious stimuli were withheld for several weeks during which time the preparations were observed in individual cages and given routine animal care only. Destruction of the ventromedial hypothalamic nuclei (Wheatley) resulted in the development of pronounced savageness. Similar although less striking results were observed following thalamic mediodorsal nucleus injury. Complete decortication gave the well-known picture of 'sham rage', whereas certain lesions of the lateral paleocortex did not precipitate angry behavior. Diminution of aggressive behavior was observed following destruction of the anterior thalamic nuclei. Lesions of the thalamic laminar nuclei resulted in transient lethargy and somnolence but with retention of ability to express 'anger.' Large rostrodorsal midbrain lesions caused a loss of faciovocal activity and 'rage', but retention of withdrawal and escape activity. Pronounced behavioral deficits (somnolence and relatively complete absence of reactivity of noxious stimuli) were manifested following lesions of the midbrain tegmentum.

Electrical properties of blood at ultrahigh frequencies. HERMAN SCHWAN (introduced by J. E. ECKENHOFF). *Robinette Fndn., Med. Clinic, Hosp. of Univ. of Pennsylvania and Dept. of Medicine, Med. School, Univ. of Pennsylvania, Philadelphia.*

Measurements of the capacity and resistivity of blood have been carried out in the frequency range from 150 to 900 megacycles. Measurements of other investigators have covered the frequency ranges above 1000 megacycles and below 100 megacycles. The total material is presented in curves to cover the range from 25–20,000 megacycles. An analysis of these curves shows the influence of various parts of the blood structure upon the electrical data. Protein molecules strongly influence the curves at frequencies above 300 megacycles. Below 300 megacycles the curves are affected by cell membranes. Equations are presented which describe the results within the accuracy of measurement. They assume that water molecules have only one relaxation time. Measurements with distilled water verify this assumption. The analysis shows that the properties of blood cannot be satisfactorily described by the introduction of polarization within the cell membranes. It is necessary to consider a distribution of electrical relaxation times. (This study was supported in part by a grant of the National Heart Institute, U. S. Public Health Service.)

Evidence that urea is excreted in human sweat by a passive process. IRVING L. SCHWARTZ, JØRN HESS THAYSEN AND VINCENT P. DOLE (introduced by HERBERT S. GASSER). *Hosp. of the Rockefeller Inst. for Med. Research, New York City.*

Following an intracutaneous injection of mecholyl (4 mg.) and procaine (3.2 mg.) in 0.5 cc. of water, the emergent sweat was collected on filter paper discs during consecutive periods of 20–30 minutes' duration. The volume of sweat was measured by weight increment and the content of urea was determined by microanalysis. The concentration of urea in the sweat always exceeded the concentration in blood; the ratio of these values (S/P) averaged 1.92 ± 0.48. Despite variation of the blood level from 5.3 mg.% (subjects on low protein diets) to 328 mg.% (oral administration of urea), the S/P ratio was unaffected. Moreover, in 2 patients with progressive uremia the same constancy of ratio was observed as the blood levels rose from 63–250 mg.%. This constancy of ratio was not due to compensatory change in sweating rate; unlike the urine/plasma ratio, the S/P ratio does not vary with flow rate. The fact that the sweat gland excretes urea in constant proportion to the blood level when the load presented to the tubular cells is increased 50-fold suggests that the mechanism of transfer does not depend upon cellular work.

Water and sodium distribution studied by simultaneous measurement of D_2O, Na^{24} and inulin spaces. I. L. SCHWARTZ, A. NELSON, P. WEISZ, M. H. MAXWELL, E. BREED AND L. SILVER. (introduced by LUDWIG W. EICHNA). *Dept. of Physiology, New York Univ. College of Medicine, New York City.*

The equilibrium volumes of distribution of D_2O, Na^{24} and inulin were determined simulta-

neously in dogs and man. In some experiments the rates of approach to equilibrium of D_2O and Na^{24} were compared. In the method an inulin prime was followed by an infusion. Cyclotron-prepared $Na^{24}Cl$ solutions were evaporated to dryness, the residue redissolved in 99.8% D_2O and injected intravenously. Whenever possible a single equilibrium blood sample was drawn for determination simultaneously of inulin, Na^{24} and D_2O spaces. The D_2O concentration exceeding natural occurrence was measured densimetrically. Initial samples were checked by the mass spectrometer. Water of frozen blood samples was obtained by sublimation and condensation under reduced pressure. Contaminating volatile impurities were burned at 750°C. The condensate was redistilled until the density remained constant and then compared with standards. Duplicate aliquots yielded identical analytic results in most instances whether combusted or not. The activities of aliquots of plasma and diluted decay standard were measured with a Geiger-Muller counter. Intracellular water (D_2O minus inulin space) averaged 433 cc/kg. (±42) in 12 men and 410 cc/kg. (±39) in 12 dogs. Intracellular sodium (sodium minus inulin space multiplied by plasma sodium concentration) averaged 17.3 mEq/kg. (±3.0) in 5 men and 11.3 mEq/kg. (±3.9) in 10 dogs. The Na^{24}/D_2O half-time equilibrium ratio was 5.5 in a human subject and averaged 3.0 in 3 dogs.

Effect of cobalt administration on dipeptidase activity of rat plasma and surviving diaphragm. THEODORE B. SCHWARTZ AND CATHERINE C. TOWBIN (introduced by FRANK L. ENGEL). *Dept. of Medicine, Duke Univ., Durham, N. C.*

Cobalt increases the rate of enzymic hydrolysis of glycylglycine (GG) by various animal tissue extracts *in vitro*. Similarly, cobalt activation of dipeptidase (GGase) activity of surviving rat diaphragm has been demonstrated (*Federation Proc.* 10: 123, 1951). This report deals with the acute effect of parenteral administration of cobalt on GGase activity. Rats were sacrificed 30 minutes after the intraperitoneal injection of cobalt sulfate, 10 μM/100 gm. of rat. Plasma GGase activity (expressed as μM GG hydrolyzed/hr/0.1 ml. plasma/ml. of hydrolysis mixture) rose from 0.36 ± 0.03 (±S.E.) to 0.72 ± 0.07. The hydrolysis mixture contained 0.02 M veronal (pH 8.0), 50 μM GG, 0.2 ml. of plasma and 0.85% NaCl to bring the final volume to 2 ml. When 1 mM Co^{++} was present in the hydrolysis mixture of both control and cobalt-injected animals, plasma GGase activity increased to 1.15 ± 0.06 and 1.37 ± 0.07, respectively. After intraperitoneal cobalt administration, dipeptidase activity (expressed as μM GG hydrolyzed/min/gm. tissue) of surviving rat diaphragm was significantly increased; mean values of 0.25 ± 0.029 were obtained in contrast to 0.10 ± 0.008 found in control animals. The significance of these results will be discussed.

Biosynthesis of cholesterol. E. SCHWENK* AND N. T. WERTHESSEN. *Worcester Fndn. for Experimental Biology, Shrewsbury, Mass., and the Fndn. of Applied Research, San Antonio, Tex.*

In our studies on cultured livers using C^{14} acetate, it was found that digitonin precipitates from the crude lipid fraction a mixture of cholesterol and other possibly related substances, some of which have a higher specific activity than the cholesterol itself. Pure cholesterol can be obtained from the mixture by dibromination. Studies on the intact animal have shown that the presence of companions in the digitonin precipitate is dependent on the time elapsed between administration of the acetate and sacrifice of the animal. In animals killed 4 hours or more after the administration of the acetate, the cholesterol is pure by digitonin precipitation alone. The cholesterol from animals killed 1 hour or less after injection shows a high concentration of companion substances. The significance of these findings and the nature of the companion substances will be discussed.

Mechanism of spinal cord regeneration in the cat. DONALD SCOTT, JR. AND C. D. CLEMENTE.* *Dept. of Anatomy, Univ. of Pennsylvania, Philadelphia.*

In a recent report, Scott and Clemente (1951) described electrophysiological evidence of regenerating intrinsic spinal cord neurons following treatment with a bacterial polysaccharide (Pyromen-Baxter). It was admitted in that report that a small number of the experimental animals had failed to show significant regeneration despite treatment, and it seemed likely that a second factor was involved in regeneration. Since that report a further series of animals has been studied and the previous findings confirmed. In addition, at the time of cord transection a measurement was made of the distance separating the proximal and distal stumps as an indication of the degree of proximation of the 2 ends of the cord. It was found that where 1 mm. or less remained between the 2 stumps at the close of the operation active regeneration followed, resulting in potentials up to 25–30% of normal. However, in those cords where the separation was 2 mm. or more no regeneration was seen beyond the site of the lesion although abortive regeneration occurred up to this point. In the latter case a considerable invasion of connective tissue into the site of the lesion was seen which was not present when close proximation of the stumps was obtained. We conclude that both treatment with the bacterial polysaccharide and close proximation of the two cut ends

of the cord are necessary for successful regeneration of spinal cord neurons in adult cats. (Aided by grants from Baxter Laboratories and Faculty Research Fund, University of Pennsylvania.)

Functional studies in dogs with prosthetic mitral valves. GORDON W. SEARLE,* SHIRLEY J. HODGINS,* SEYMOUR A. HORWITZ* AND HAROLD C. WIGGERS. *Dept. of Physiology and Pharmacology, Albany Med. College, Union Univ., Albany, N. Y.*

Single-piece polyethylene valves, designed to provide normal or abnormal valvular performance, have been surgically implanted in the mitral ring of the hearts of a series of dogs. Some of these dogs have been employed for cardiopulmonary studies in order to quantitate the effectiveness of the implanted units. From the standpoint of appetite, growth, spirit and spontaneous activity, these valve dogs appeared normal. The responses to the stress of hypoxia, in terms of heart rate, arterial blood pressure, pulmonary ventilation and arterial oxygen saturation, have been measured in several dogs 9 to 24 months after operation for comparison with those in an untreated control group. These measurements were made before, during and after 20-minute periods of both 10% and 8% oxygen (in nitrogen) inhalation, after the dogs had been trained to the procedures. In general, the patterns of response in the valve and in the control dogs were not noticeably different.

Mechanism of reduction in electrolyte and water excretion resulting from ureteral pressure elevation. EWALD E. SELKURT, MARTIN BRANDFONBRENER* AND HERMAN M. GELLER.* *Dept. of Physiology, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

Elevation of ureteral pressure in dogs in two stages (36 and 52 cm. above control) results in slight modification only of the PAH clearance. Glomerular filtration rate decreases to 88% of control at the highest ureteral pressure, so that the filtration fraction decreases. Much more pronounced effects are noted on electrolyte and water excretion. Potassium excretion declines to 80% of control, sodium to 50%, and urine volume to the same degree. Evidence is supplied that the changes in electrolyte and water clearance are probably the result of small changes in filtration rate, for when arterial perfusion pressure is increased by means of a pump to the kidney in which ureteral pressure has been elevated, filtration rate tends to be restored, and the decreases in electrolyte and water excretion are restored to normal.

Electrophoretic study of urinary protein in the rat. A. SELLERS,* S. ROBERTS, I. RASK,* S. SMITH, III,* J. MARMORSTON AND H. GOODMAN.* *Inst. for Med. Research, Cedars of Lebanon Hosp., Dept. of Medicine, Univ. of Southern California School of Medicine, and Dept. of Physiological Chemistry, School of Medicine, Univ. of California at Los Angeles.*

The rat normally excretes an average of 0.1-0.8 mg. of protein in its urine/hour. In the hour following the intraperitoneal injection of 4 Goldblatt DU of hog renin the excretion of protein averages 31.7 mg. Electrophoretic analysis indicates that the major portion of the urinary protein normally excreted by rats of the Slonaker-Addis strain is similar to serum α and β globulins. Occasionally, albumin and γ globulin may be present in small quantities. Electrophoretic patterns of urinary protein following renin administration differ strikingly from those of normal urinary protein, but closely resemble normal serum protein patterns. Thus, proteins having electric mobilities similar to serum albumin, α globulin, β globulin, and γ globulin are present in the urine in proportions similar to those existing in normal rat serum.

Role of somatotrophic hormone (STH) in body defense against infection. HANS SELYE. *Inst. of Medicine and Experimental Surgery, Univ. of Montreal, Montreal, Canada.*

Earlier experiments (SELYE, *First Annual Report on Stress*—Montreal: Acta Inc., Medical Publishers, 1951) had shown that in adult rats heavily overdosed with cortisone, there is a great tendency to develop fatal 'spontaneous infections,' apparently due to a considerable diminution of the body's resistance to normally saprophitic microorganisms. In the adult rat, these infections tend to localize primarily in the lung and on the pleural surface, although occasionally microbial foci are also detectable in the liver, spleen, kidney and other locations. Treatment with STH diminishes or abolishes all these manifestations of infection and help the adult rat to withstand otherwise fatal doses of cortisone. Recent studies on young rats (weighing 30–40 gm.) revealed that in them comparatively small doses of cortisone (1.25 mg/day, during given 2 weeks) suffice to produce infections, with great regularity. Unlike in adults, here, the liver is the site of predilection for the localization of such infections and at the same time multiple foci of hepatic necrosis develop. Both the infections and the hepatic necroses can be prevented by simultaneous treatment with 1–2 mg. of purified STH (Armour). The probable rôle of STH in the body's normal defense against infections will be discussed. (Aided by a grant of the National Research Council of Canada.)

Infusion of dextran solution into normal dogs. ROBERT E. SEMPLE (introduced by CHARLES H. BEST). *Banting and Best Dept. of Med. Research, Univ. of Toronto, Toronto, Canada.*

Dextran (6% solution) equal to about 25% of control plasma volume was infused into normal dogs. The animals were observed until dextran was no longer detectable in the plasma. Urine was analyzed for dextran and observations made on the volume of circulating blood and plasma and the concentration of red cells, proteins and electrolyte in the plasma. Dextran left the circulation rapidly at first and then more slowly. The plasma contained 60%–80% of the amount given one hour after the infusion terminated, and from 32%–55% after 6 hours. Thereafter the dextran disappeared at about 1.5%/hour. There was a 6- to 8-hour period of anuria immediately after the infusion, and the total urine for 24 hours was small in volume and accounted for about 40% of the infused material. The circulating plasma increased immediately after infusion by a volume that varied from 70%–90% of that infused, and remained significantly greater than that of controls for over 24 hours. The potassium and the proteins of the plasma were diluted by the volume of the infusion. There was no evidence of any transfer of potassium from cells to extracellular fluid. These results indicate that, in the normal dog, a dextran infusion produces a plethora which persists for over 24 hours. The subsequent reduction in plasma volume is accompanied by a loss of dextran from the plasma, and the excretion of some, but not all, in the urine. A transfer of dextran from the plasma to the interstitial fluid or to the cells is indicated and is being investigated. (Supported by Defence Research Board, Canada.)

Degradation of desoxypentosenucleic acid by ultraviolet irradiation. MARIA SERAYDARIAN,* ATTILIO CANZANELLI AND DAVID RAPPORT. *Research Lab. of the Boston Dispensary and Dept. of Physiology, Tufts College Med. School, Boston, Mass.*

It has been previously shown that nucleic acids and various derivatives lose their specific ultraviolet absorption spectra when irradiated with ultraviolet light for periods of about 18 hours. In the present study a solution of highly polymerized desoxypentosenucleic acid was irradiated for varying periods and changes in the specific absorption spectrum, electrometric titration and inorganic phosphate were observed. After 2 hours of irradiation a 17% reduction in the optical density at 260 mμ was found. No change in the electrometric titration curve occurred nor did any inorganic phosphate appear at this time. A 35% reduction in the optical density resulted after 4 hours irradiation, at which time a perceptible increase in titration occurred in the range of secondary phosphoryl dissociation. No inorganic phosphate appeared. With long periods of 18 hours or more almost complete loss of the specific absorption resulted, associated with a marked increase in the alkali uptake by secondary phosphate. About 30% of the total phosphate appeared in the inorganic form. Thus the light absorbing purine and pyrimidine rings seem more susceptible to disruption by ultraviolet irradiation than the phosphate linkages, for the secondary phosphoryl dissociation just begins to appear when roughly 35% of the rings are broken. When the rings are almost completely destroyed we have in addition a considerable breakdown of the phosphate to the inorganic form.

Effect of endocrine factors on experimental renal hypertension. ROGER W. SEVY* AND GEORGE E. WAKERLIN. *Dept. of Physiology, Univ. of Illinois College of Medicine, Chicago.*

Five normotensive dogs and 12 renal hypertensive dogs were given one or more courses of intramuscular injections of crude beef anterior pituitary extract (0.5–1.0 gm. of fresh tissue equivalent per kg. of body weight daily) for 1 to 6 months. There was no significant change in blood pressure in the normotensive group, although one of these animals appeared to be protected against the development of experimental renal hypertension following subsequent renal artery constriction. In the renal hypertensive group there was a significant reduction in blood pressure, repeated courses of treatment being more effective than single courses of treatment. The antihypertensive effect was not correlated with alterations in the renal clearances of creatinine or PAH, or with alterations in the response to the acute pressor action of renin. Preliminary results indicated that ACTH did not reproduce the results obtained with the crude extract. An immunologic mechanism is suggested. In 3 dogs the administration of desoxycorticosterone acetate (5.0 mg/kg. of body weight daily and intramuscularly) for 2 months preceding, and up to 1 month after, renal artery constriction did not hinder, and may have enhanced, the development of experimental renal hypertension. In 2 of these dogs there was no detectable increase in renin secretion 1 month after renal artery constriction; renal renin concentration was markedly reduced.

Comparison of dye dilution cardiac output with a rotameter and the pressure pulse contour method. OSCAR W. SHADLE,* THOMAS B. FERGUSON* AND DONALD E. GREGG. *Dept. of Cardio-Respiratory Diseases, Army Med. Service Graduate School, Washington, D. C.*

The constant flow photometer of Friedlich *et al.* for continuously recording the concentration of Evans Blue Dye in whole blood has been used in measuring the cardiac output. Twenty-two technically satisfactory comparisons have been made

between this method and a rotameter in 3 preparations; an artificial pump system, in 2 dogs with the rotameter in the pulmonary circuit, and in 4 dogs with the rotameter tied in the thoracic aorta. In the latter experiments all aortic branches proximal to the rotameter were securely ligated and an estimated coronary flow of 5% of the total cardiac output was added to the measured rotameter flow. Flows ranging from 726–2274 cc/min. were compared. The largest percentage variation between the 2 methods was +30%, the remaining 21 comparisons had extremes of variation of +12% and −10%. In another group of 28 experiments in anesthetized intact dogs carotid pressure pulse contours were optically recorded bracketing or simultaneous with optical tracings of the dye dilution curve. The pressure pulse contour outputs were averaged over a 12- to 15-second interval in records with regular pulses, heart rates under 200 and diastolic pressures less than 120 mm. Hg, for comparison with the dye dilution output. Here, percentage variation between outputs was greater with extremes of variation of +34% and −26%.

The ultra-violet spectra and neurophysiological effects of 'veratrine' alkaloids. ABRAHAM M. SHANES. *Natl. Insts. of Health, Bethesda, Md.*

Comparison of the ultra-violet spectra and the neurophysiological effects of the isolated alkaloids veratridine, cevadine or cevine (all kindly provided by Dr. O. Krayer) with those of the crude drug 'veratrine' indicates that the latter is approximately a 1:1 mixture of the first 2 alkaloids on a weight basis. Three distinct absorption peaks by veratridine at 217, 263, and 292 mμ (ϵ = 20.5×, 12.3×, 6.3 × 10^6 cm^2 mol^{-1}, respectively) arise from the veratric acid moiety and constitute positive identification. The difference between the veratrine and veratridine spectra is accounted for by cevadine, which can be identified by one absorption maximum at 222 mμ (ϵ = 8.7 × 10^6 $cm.^2$ $mol.^{-1}$) attributable to its tiglic acid content. Cevine gives a considerably weaker end absorption; hence, although it is the major component of the veratridine and cevadine molecules, it does not contribute to the spectrum. The prolongation of the negative after-potential in the squid giant axon, crab leg nerve, and frog sciatic is increased by the drugs according to the following series: cevadine < veratrine < veratridine. The decline of the negative after-potential is a simple exponential in the pure alkaloids; the time constant is 5–10 times greater in veratridine than in cevadine, hence the individual effects of the alkaloids are readily identified in the mixture. Both cevadine and veratridine can cause a bout of repetitive activity following the application of single stimuli. These alkaloids were also observed to enhance the subthreshold oscillatory behavior of the giant axon.

Gastric and esophageal temperatures in man. HERBERT SHAPIRO AND EMERY K. STONER.* *Dept. of Physical Medicine, Univ. of Pennsylvania, Philadelphia, Pa.*

A copper-constantan thermocouple, mounted on a small plastic rod, is swallowed, and then pushed into the stomach. Temperatures in normal adults were recorded with a Brown automatic potentiometer. By pulling the thermocouple out of the stomach and moving it along the esophagus, the esophageal temperature could be readily recorded. The temperature of the stomach was about 37°C. (approximately the same as the deep rectal temperature) but in the thorax, from the cardiac orifice on toward the region of the epiglottis, a decline was observed, totalling approximately 0.5°C. The epiglottidean region is of particular interest, for a rhythmic change in temperature is observable there corresponding to the respiratory rhythm. Cold air entering the bronchi cools the thermocouple, while air making its exit from the lungs warms the thermocouple. The effects of deep vs. shallow respiration are demonstrable, and the temperature may fall to 34.5°C. On emergence into the oral cavity the temperature rises again.

Effect of sublethal total body x-radiation on susceptibility to certain microbial agents. I. L. SHECHMEISTER, L. J. PAULISSEN AND M. FISHMAN (introduced by E. W. DEMPSEY). *Dept. of Bacteriology and Immunology, Washington Univ. School of Medicine, St. Louis, Mo.*

The susceptibility of sublethally irradiated mice to infection with *Salmonella enteritidis*, *Escherichia coli*, influenza virus and certain other microbial agents was investigated. The x-ray dose, the post-irradiation time, the concentration of the microörganisms and the route of administration were treated as variables. In the experiments reported here, x-ray doses were varied from 50 r to 350 r, the latter being the largest dose that was consistently sublethal to our strain of mice. Exposure to 350 r, when followed by challenge with the organisms in question, showed the mice to have an increased susceptibility to the effects of the several agents used in this study. For example, when determined 1–5 days after irradiation, the LD_{50} of *S. enteritidis* for normal mice was about 1 × 10^7 organisms administered intraperitoneally, while the LD_{50} for the irradiated animals was of the order of 1 × 10^2 organisms. Similarly, while normal animals were not affected by intraperitoneal administration of *E. coli* (when mortality was used as the criterion of response), the irradiated mice succumbed to the inoculation of a comparable number of organisms. Increased susceptibility of irradiated mice to microbial agents was shown to be a function of the post-irradiation time and the x-ray dose. The initial experiments attempting to elucidate the possible mechanism(s) involved in

this phenomenon consisted, in part, of studies healing with the effect of ionizing radiation on phagocytosis and on the response to immunization.

Transcapillary movement of an isotope from a nonuniformly mixed circulatory pool. C. W. SHEPPARD AND WARD SANGREN.* *Biology Div. and Mathematics Panel, Oak Ridge Natl. Lab., Oak Ridge, Tenn.*

We have investigated the data of Walker and Wilde (*Am. J. Physiol.* 163: 759, 1950) on the disappearance of K^{42} from the circulation of the rabbit. Here the circulatory level of isotope changes so rapidly that no semblance of a uniformly mixed pool exists. Our analysis applies where a large fraction but not all of the isotope is removed on one circulatory passage. It is also confined to the case of a large extravascular pool and to events early in time where minimal return flow of tracer occurs. In the case of observations on the inflow to and outflow from a capillary bed, using semilog plots of concentrations, events upstream are repeated downstream but displaced in time by the mean circulation time between the points of observation. Downward displacement occurs by the product of the circulation time and the mean exchange rate between the points. Smearing occurs as a result of the dispersing action of variable path lengths in the capillary bed. For the circulation as a whole the time relations consist of the sum of a periodic (circulatory mixing) component and an aperiodic component. The latter is initially nearly straight on semilog coordinates and extrapolates back to the blood volume dilution when injection and sampling points are close together. When they are separated correction must be made for time delay and loss of tracer between. The initial slope gives the over-all mean exchange rate, plus a small contribution which takes into account the small effect of variations in circulation rate.

Role of reticulo-endothelial system in uptake of plasma phospholipides. M. L. SHORE* AND D. B. ZILVERSMIT. *Div. of Physiology, Univ. of Tennessee, Memphis.*

Since the liver is the principal organ involved in the uptake of plasma phospholipides (ENTENMAN *et al. J. Biol. Chem.* 15: 166, 1946), it is of interest to know whether the reticuloendothelial system in the liver is concerned with this process. Plasma phospholipides occur in colloidal complexes, thus they might be removed from the circulation by Kupffer's cells. We measured the disappearance of P^{32}-labeled plasma phospholipides from the plasma of normal dogs. Two days later half of these animals were injected with 100 ml. of 8% India ink before the same radioactive plasma was again administered to this group and their controls. The average 30 minute bromsulfalein retention in the normal animals was 6.3%; in the animals receiving India ink, it was 52%. The turnover time of plasma phospholipides varied from 9 to 17 hours in the control animals, and from 10 to 17 hours after India ink administration. Statistical analysis showed no significant difference between these turnover times. If bromsulfalein retention is a measure of the function of the reticulo-endothelial system (MILLS AND DRAGSTEDT. *Proc. Soc. Exper. Biol. & Med.* 34: 228, 1936), the above data suggest that this system is not involved in the removal of plasma phospholipides. However, if India ink produces circulatory disturbances in the liver (BRAUER AND PESSOTTI. *J. Pharmacol. & Exper. Therap.* 97: 358, 1949), a different interpretation of our results is required. (Supported by U. S. Public Health Service.)

Cross-resistance to hemorrhagic shock in rats made resistant to drum-trauma; relation to hepatic VDM inactivation mechanisms. EPHRAIM SHORR, SILVIO BAEZ, DELILAH B. METZ* AND BENJAMIN W. ZWEIFACH. *Dept. of Medicine, Cornell Univ. Med. College and New York Hosp., New York City.*

We have previously shown that resistance to drum shock, acquired by repeated exposures to sublethal trauma in the Noble-Collip drum was associated with enhancement of hepatic VDM (ferritin) inactivating mechanisms. The present experiments indicate that rats made resistant to trauma also withstand a graded hemorrhage lethal to control rats, preserving their hepatic VDM inactivation system following a period of hypotension sufficient to lead to its deterioration in controls. Graded hemorrhage was carried out by self-infusion reservoir technic, blood pressure being reduced to 70 mm. Hg for 1 hour, 50 mm. Hg for 1 hour, then kept at 35 mm. Hg for 2 hours, after which all of the blood was returned to the animal. Mortality after 24 hours was 61% for controls, 20% for resistant rats. The latter required withdrawal of more blood to achieve equal hypotensive levels, and took up less blood from reservoir during the period of drastic hypotension (35 mm. Hg). In another series, blood bioassays were made and livers removed for study at end of the drastic hypotensive period. Blood and liver wash from controls contained VDM. There was profound deterioration to total loss of VDM inactivating capacity. Although blood from resistant rats also contained VDM, the livers retained normal capacity to inactivate VDM aerobic on incubation *in vitro*. A relation is suggested by these data between resistance to hemorrhagic shock and preservation of hepatic inactivation systems.

Effect of negative pressure breathing on renal function in human beings. H. O. SIEKER,* O. H. GAUER* AND J. P. HENRY. *Aero Med. Lab.,*

Wright-Patterson Air Force Base, Dayton, Ohio.

Previous studies have suggested that receptor areas located in the low pressure side of the cardiovascular system within the thorax appreciate changes in blood volume and alter urine flow (HENRY AND GAUER, *Federation Proc.* 10: 62, 1951; GAUER *et al.*, *Am. J. Physiol.* in press). Decreasing the blood volume in the thoracic viscera by positive pressure breathing is associated with oliguria in humans (DRURY, HENRY AND GOODMAN, *J. Clin. Investigation* 26: 945, 1947). Following 5-hour control periods recumbent subjects pressure breathed at −15 cm. water for 45 minutes. Urine flow, pH, sodium excretion and filtration rate were measured. Blood pressure, pulse rate and respiration rate were also followed. During negative pressure breathing the urine flow showed an average increase of 350%. The urine pH during the period was unaltered and the urine sodium concentration decreased an average of 72%. The total sodium excreted during negative pressure breathing increased only 16% and in 2 of 5 subjects, the total sodium did not increase or fell below control levels. The pulse rate, blood pressure and respiration rate were unaltered or inconsistently changed. The inconsistent change in respiration rate, the unaltered urine pH and the slight average increase in total urine sodium excretion make alkalosis an unlikely cause for the marked diuresis. It is suggested that the diuresis is the result of engorgement of receptor areas in the thorax which mediate changes in renal function through the endocrine glands and renal hemodynamics.

Auricular excitability of intact dog heart. RAJINDER S. SIKAND AND LOUIS H. NAHUM. *Lab. of Physiology, Yale Univ. School of Medicine, New Haven, Conn.*

A technique of sterile implantation of electrodes permitted observations to be made repeatedly over a period of weeks to determine the excitability of the left and right auricles in dogs with closed chests. The method used for this investigation was the same as that for determining the excitability of the ventricles which was described in the preceding paper. A steady state of Nembutal anesthesia was maintained throughout the experiments. In the same animal, at the same heart rate, the absolute refractory period was the same for both auricles, but differences between the auricles in the relative refractory periods and the threshold levels were encountered. Repeated observations revealed a constancy of results in any one animal. However, in different animals, even at the same heart rate, the right and left auricles showed differences in the duration of the refractory periods as well as in the threshold levels. The relative refractory period exhibited an evenly declining curve. Supernormality was not encountered during the diastolic threshold period which appeared as a smooth plateau. A comparison of the auricular with the ventricular excitability cycles showed great similarity relating to the shapes of the curves, i.e. differences between the two sides on recovery, evenly declining relative refractory periods, and smooth diastolic plateaus.

Effect of relative body weight on the normal electrocardiogram. ERNST SIMONSON AND ANCEL KEYS. *Lab. of Physiological Hygiene, Univ. of Minnesota, Minneapolis.*

Present information about the effect of relative body weight on the ECG is inadequate and limited mainly to the electrical heart position. Statistical analysis according to relative body weight was made for 48 items in the standard and unipolar limb leads and CF4 of 157 men from 18 to 25 years and 222 men from 45 to 55 years, all of whom were found to be clinically healthy in detailed examinations. In general, the influence of relative body weight was more prominent in the older than in the younger men, both in regard to the number of items with statistically significant age trends (12 in older men, 4 in young men) and to the magnitude of the difference. In some items there was a linear relationship with the relative body weight over the entire range, but in a few items there was a maximum or minimum in the men of intermediate relative body weight. There was a pronounced tendency toward the horizontal electrical heart position with increasing body weight but many effects of relative obesity on the ECG are not explained on the basis of heart position. There are similarities between the effect of age and relative body weight on the ECG, but the two factors are not identical and must be considered separately. Properly defined standards for the electrocardiogram should be based on age and relative body weights. (Supported by a grant from the U.S. Public Health Service.)

Distribution of infused sodium bicarbonate solution in man. R. B. SINGER, J. K. CLARK, A. P. CROSLEY, JR. AND E. S. BARKER (introduced by D. W. WILSON). *Depts. of Research Medicine and Medicine, Univ. of Pennsylvania, Philadelphia.*

The apparent volume of distribution (V) of sodium bicarbonate in 13 normal subjects and convalescent patients was estimated from serial acid-base data of blood and urine, for 2 hours following the infusion of 2.4 mEq/kg. of a 7.5% solution in 10 minutes. V averaged 14% of body weight at the end of the infusion, 22% at 10 minutes, 29% at 30 minutes, 35% at 120 minutes. The initial rise in V indicates that sodium and bicarbonate ions diffuse with great rapidity (time for 50% equilibrium, 8 minutes) from plasma into a volume twice as great as the currently accepted estimate of

extracellular fluid, 16% of body weight. It is suggested that this compartment into which sodium does, but inulin does not, diffuse may be connective tissue, which is high in sodium and might equilibrate rapidly with this ion in interstitial fluid. The later rise in V is taken to represent sodium transfer to typical high-potassium intracellular compartments, such as muscle. This transfer is generally slow and incomplete, although in 3 of 15 tests most of the retained alkali had disappeared from extracellular fluid at 2 hours. V at 120 minutes becomes 75% of body weight when *no* allowance is made either for urinary loss of alkali, or for rise in blood buffer anions other than bicarbonate. This discrepancy accounts for the prevalent notion that sodium bicarbonate is distributed uniformly throughout body water.

Effect of 'Benemid' (*p*-(di-n-propylsulfamyl)-benzoic acid) on urate clearance and other discrete renal functions in gouty subjects. J. H. SIROTA, T. F. YÜ* AND A. B. GUTMAN. *First Med. Service, Mount Sinai Hosp., New York City.*

Simultaneous renal clearances of inulin, urate, *p*-aminohippurate, sodium, potassium and chloride were determined in 10 gouty subjects before and for 2 hours after oral administration of 2.0 gm. 'Benemid.' In 6 of these phosphate clearances were also studied; in 2 ammonium, hydrogen ions, urine and blood pH. In 3 additional subjects these studies were extended for 24 hours. The subjects had no clinically evident renal damage, but most had a slight to moderate depression of GFR (range: 73–124, mean: 96.7 ml/min/1.73 m.²) and RPF (range: 307–596, mean: 475 ml/min/1.73 m.²). There was a consistent and striking increase in urate clearance, $C_{urate}/C_{inulin} \times 100$ rising from a pre-medication mean of 8.18 (range 5.02–10.3) to an average peak of 33.4 (range 11.8–54.7). This increase was usually evident within 40 minutes after oral 'Benemid' and persisted for 24 hours. A slight to moderate decrease in C_{PAH} was also noted, $C_{inulin}/C_{PAH} \times 100$ rising from a control mean of 21.2 (range: 15.7–25.3) to a mean of 33.3 (range: 17.5–82.5) after 'Benemid.' There was no significant change in inulin clearance, electrolyte excretion or other renal functions tested. 'Benemid' is a potent and apparently highly selective uricosuric agent inhibiting tubular reabsorption of urate from the glomerular filtrate.

Conduction time in the central nervous system and peripheral nerve excitability in relation to adrenal cortical function. ALAN SLOCOMBE,* HUDSON HOAGLAND AND JULIUS PRAGLIN.* *Worcester Fndn. for Exptl. Biology, Shrewsbury, Mass.*

Cathode ray oscillograph records of the electrical response of the rat somatic sensory cortex have been made following tactile and electrical stimulation of the foot. Adrenalectomized rats kept in good condition on salt show, in comparison to normal rats, increases in the latency of the response by 19% and this slowing of conduction time is significant ($P < 0.01$). The simultaneous recording with two cathode ray beams of the cortical response and that of the sciatic nerve indicate that the increase in conduction time is centrally determined. The excitability of the rat sciatic nerve is decreased about 200% ($P < 0.005$) following adrenalectomy as measured by Hill's excitation constant. The administration of 0.25 cc. of Upjohn's lipoadrenal extract restores the excitability nearly to normal within 3 hours and these findings confirm changes in sciatic chronaxie in adrenalectomized animals earlier reported by Chauchard. (This work has been aided by a grant from the Physiology Section, U. S. Public Health Service, Federal Security Agency and by a contract with the Office of Naval Research.)

Effect of x-irradiation upon food and water intake and body weight. D. E. SMITH, E. B. TYREE,* H. M. PATT AND N. BINK.* *Argonne Natl. Lab., Chicago, Ill.*

Single, total-body x-irradiations (25–10,000 r) of rats are immediately followed by anorexia, weight loss and polydipsia. Recovery is complete in 2–4 days after 25–500 r. Following 800 r (80% lethal) anorexia and weight loss persist until death in rats succumbing within 7 days but only 4–5 days in those surviving longer. Twenty-eight-day survivors show lasting recovery, whereas nonsurvivors suffer a sharp fall in food intake beginning on *days 10–12* and lasting until death. Severe irreversible anorexia and weight loss follow 1000–10,000 r. Polydipsia occurs on *days 1 and 2* over the entire dosage range and again on *day 5* in survivors of lethal exposures. The degree of the responses depend on radiation dosage. The magnitude of the weight loss depends on the degree of anorexia. Single, partial-body (head, chest, abdominal quadrants, hind legs and tail) exposures to 800 r, 1200 r and 5000 r elicit similar responses, the degree and duration of which depend upon radiation dosage and the part irradiated. Exposure of the abdomen and the hind legs-tail produces the greatest and least effects, respectively. Cysteine pretreatment combined with force-feeding of a semi-fluid diet greatly reduces post 800 r (total-body) weight loss. Force-feeding or subcutaneous injection of protein hydrolysate (Amigen)-dextrose greatly reduces weight loss after 250 and 500 r. Force-feeding of either semi-fluid diet or Amigen-dextrose or subcutaneous injection of Amigen-dextrose is followed by death of all rats within 4 days after 800 r.

Cardiovascular changes accompanying increasing blood levels of potassium. EDWIN L. SMITH, RUSSELL A. HUGGINS, RUTH SAXTON* AND DORIS GEISLER.* *Dept. of Physiology, Univ. of Texas, Dental Branch, and Dept. of Pharmacology, Baylor Univ. College of Medicine, Houston, Tex.*

The dogs were anesthetized with morphine sulfate, 10 mg/kg. subcutaneously, followed in about 15 minutes with 10 mg/kg. of sodium pentobarbital intravenously. The ureters were tied and isotonic potassium chloride infused into a femoral vein until the death of the animal. Pressures were obtained from various parts of the circulatory system with Hamilton manometers and simultaneous E.C.G. records (lead II) were taken on the same record as the pressures. The mean pressure tended to rise during the first portion of the infusion and then leveled off, whereas the cardiac output remained fairly constant; thus the increased mean pressure was the result of increased peripheral resistance. The mean pressure remained high, falling precipitously just before the dog died. Deterioration of the E.C.G. pattern is not accompanied by observable effects on mean and right and left ventricular pressures. Filling pressure of the ventricle changed but little throughout the infusion. During intraventricular block electrical activity of the cardiac muscle occurred, but was not necessarily followed by a measurable mechanical event in the aorta. When pressure was recorded in the left ventricle every electrical complex was accompanied by a pressure rise.

Influence of ethyl alcohol and testosterone on the respiration of rabbit liver and kidney slices. THOMAS C. SMITH* AND CHARLES D. KOCHAKIAN. *Oklahoma Med. Research Inst. and Hosp., Oklahoma City, Okla.*

The respiration of liver and kidney slices from fasted rabbits was studied by the Warburg method using Krebs Ringer-phosphate medium and a temperature of 37.5°C. The addition of ethyl alcohol or testosterone in alcohol solution inhibited or stimulated respiration depending upon concentration of the compound and the tissue under observation. In low concentrations, ethyl alcohol exerted a stimulative action, whereas in higher concentrations it exerted an inhibitory action on both liver and kidney slices. Testosterone inhibited the respiration of kidney slices, but had a variable effect on the respiration of liver slices. The response of rabbit tissues to ethyl alcohol is similar to that of rat tissues (FONDAL AND KOCHAKIAN, *Proc. Soc. Exper. Biol. Med.* 77: 823, 1951). However, greater amounts of testosterone are required to produce changes comparable with those seen in mouse (KOCHAKIAN, *Proc. Soc. Exper. Biol. Med.* 77: 666, 1951) and rat (unpublished) tissues; furthermore, the results are more variable. (This investigation was aided by a grant from the American Cancer Society.)

Monosynaptic and multisynaptic arcs within the cerebellum. RAY S. SNIDER AND MARTYN A. GOLDMAN.* *Dept. of Anatomy, Northwestern Univ. Med. School, Chicago, Ill.*

Monosynaptic and multisynaptic arcs within the cerebellum have been studied by applying electrical pulses to various cerebeller afferents and recording the altered activity in a cerebellar efferent system. Cathode ray tracings of responses within the brachium conjunctivum was the efferent pathway most frequently investigated. Afferent volleys were applied to the fibers of the brachium pontis, brachium restiformis, inferior olive, and spinocerebellar tracts. If a single threshold pulse be applied to afferent fibers, then a complex wave with 3 peaks results. The first wave has a latency (to rising phase) of approx. 1 msec. and probably represents the activity of a collateral from afferent fiber to central cerebellar nuclei, thence out brachium conjunctivum. The effect of repetitive shocks on this by-pass of cerebellar cortex is being studied. The second wave, with latency of approx. 2.5 msec., is interpreted to represent the activity of impulses passing through the cerebellar cortex. Rarely, the second wave has a notch on descending limb, the origin of which has not been identified. The third wave, with latency of 8–10 msec., probably indicates activity of relay systems through the reticular formation. Occasionally a wave of opposite sign appears between second and third waves. The effect of various drugs on these waves is being investigated.

Action of meperidine during labor. FRANKLIN F. SNYDER. *Depts. of Obstetrics and Anatomy, Harvard Univ., Boston, Mass.*

Meperidine exerts a definite influence upon the outcome of labor in rabbits following administration at the time of parturition. Increase in the incidence of stillbirths and increase in the occurrence of prolonged labor afford evidence of the effect upon the uterine expulsive mechanism. Observations on the spontaneous birth of 650 rabbit fetuses following meperidine show clearly that increase in the dosage increases the risk of death for the fetus during expulsion through the birth canal. Thus, following a single injection of 40 mg/kg. of meperidine subcutaneously at the time of labor, 30 or 31 days, fetal mortality increased to 15%, or definitely greater than that noted in uninjected controls; and labor was prolonged for a period of hours or days in 11% of the animals. At a total dosage level three times as great, i.e. three injections of 40 mg/kg. in the course of 24 hours, further increase in stillbirths

was noted, amounting to 27%; and in 23% of the animals labor was prolonged. The rapid escape of meperidine from the maternal circulation into the fetus is demonstrable by direct observation of breathing fetuses within the unopened uterus following laparotomy in a saline bath. Fetal respiratory movements are strikingly depressed or temporarily abolished immediately after intravenous injection of 5 mg/kg. of meperidine into the mother. Recovery from the respiratory depression is noted, but prolongation of the period of depression is observed after repeated doses of the drug. (Aided by Research Grant, U. S. Public Health Service.)

Comparative metabolism of neural regions and cells. J. SNYDER,* L. G. ABOOD AND R. W. GERARD. *Dept. of Physiology, Univ. of Chicago, Chicago, Ill.*

Separated grey cortex is some 50% more active metabolically than is cord white (ABOOD AND GERARD, *Federation Proc.* 10: 3, 1951, and *Am. J. Physiol.*, in press). Rat cortex can be sliced into 0.2 mm. sheets; and a plexiform layer, a purkinje layer, and a layer of white matter can be separated. The latter exhibits oxidation, glycolysis and other metabolic rates a third lower than the other two. Each layer can be further dissected into a bit of microglia (plexiform), pyramidal neurones (purkinje), neuroglia (purkinje or white) or fiber tracts (white). Despite differences in cell population, single bits (about .1–.2 mg.) gave Q_{O_2} values of 10–12 and $Q_{CO_2}^{N_2}$ values of about 40—figures in agreement with findings in tissue culture. Supraoptic and paraventricular nuclei, isolated by dissection under the microscope, showed respiratory and glycolytic activity comparable to other hypothalamic regions. Results on the amount of intracellular localization of enzymes, by chemical or histochemical methods, will be reported. Preliminary results, e.g. confirm E. Sharrer's evidence of a high phosphatase activity in these neurocrine cells; while succinic dehydrogenase is seen histochemically to be uniformly distributed in all neurones and glia examined, and in the cytoplasm (mitochondria). (Supported by ONR contract.)

New method of inducing repeated short bursts of ventricular cardiac arrhythmias in dog. A. SOKALCHUK, R. E. DAILEY AND J. N. CROWDER (introduced by J. W. STUTZMAN). *Research and Development Div., Smith, Kline and French Labs., Philadelphia, Pa.*

Dogs are anesthetized with a thiopental-barbital mixture. Caffeine sodium benzoate, 100 mg/kg., is injected intramuscularly. Left chest is opened under interrupted positive pressure (reasonably constant source) artificial respiration (maintained at a rate of 60–70/min.). A critical level of hypoxia is achieved; i.e. volume exchange is reduced to such a point that minimal, continuous, reflex muscular attempts of respiration are just produced. Atelectasis should be promptly corrected if it occurs. Mean arterial blood pressure and muscular attempts at respiration are recorded. The ECG is continuously observed on a cathode-ray oscilloscope; controls and significant changes are permanently recorded. Both control and test animals are challenged every 5–10 minutes with a selected dose of epinephrine intravenously. Myocardial irritability threshold is assigned to that dose of epinephrine that produces a definite but short (5–10 beats) burst of idioventricular rhythm. This threshold remains reasonably constant for 2- to 3-hour periods. Antiarrhythmic agents cause marked elevations in threshold of myocardial irritability as indicated by multiple increases in control dose of epinephrine required to elicit a threshold response. By this method, doses of 0.5–5.0 mg/kg. intravenously of 'Dibenzylene' (*n* - (phenoxyisopropyl) - N - benzyl - β chlorethylamine) and doses of 50 mg/kg. intravenously of procaine amide produce comparable elevation of the epinephrine induced arrhythmia threshold. The brief duration of action of procaine precludes an accurate determination of peak degree of activity; however, significant degrees of protection are observed in doses of 2.5–5.0 mg/kg. intravenously.

Energy relations in human red cell cation transport. A. K. SOLOMON (introduced by A. BAIRD HASTINGS). *Biophysical Lab., Harvard Med. School, Boston, Mass.*

From unpublished measurements of the chloride concentration in red cell and plasma at equilibrium, the estimated potential inside the red cell is −9.9 mv relative to the plasma. Using this figure, and the red cell and plasma Na and K concentration (red cell K, 94.2 mEq/l. cells; exchangeable Na, 6.29 mEq/l. cells; plasma K, 4.65 mEq/l.; plasma Na, 143.6 mEq/l. plasma; red cell water, 66.1%; plasma water, 93.5%) it can be calculated that at 37°C. ΔF is 1640 cals/mol. for the entrance of K, and −1720 cals/mol. for the entrance of Na. These calculations assume that the activity coefficient inside the cell is equal to that outside. If it is further assumed that energy is only consumed by the inward transport of K and the outward transport of Na (and that no energy is made available from the outward K transport and the inward Na transport) an approximate figure for the energy required to maintain the cation flux can be obtained. Since 1.67 mEq. K and 3.08 mEq. of Na are transported through the cell wall/hour/liter of cells, 2.8 cals/hour are required for the K transport and 6.0 for the Na, making a total of 8.8 cals/liter of cells/hour. Since 2.33 mm. glucose are consumed/hour/liter of red cells there is provided (on the basis of

48,000 cals/mol. available from the anaerobic metabolism of glucose) 110 cals/hour/liter of red cells. In other words of the 48,000 cals/mol. available from glucose metabolism, only about 3800 cals/mol. are required for cation flux.

Distribution of injected radioactive Menadione P. F. SOLVONUK,* L. B. JACQUES AND J. W. T. SPINKS.* *Depts. of Physiology and Chemistry, Univ. of Saskatchewan, Saskatoon, Canada.*

One mg. and 0.1 mg. doses of 2-methyl-1,4-naphthoquinone (Menadione) labeled with C^{14} in the methyl group were injected intramuscularly into mice. The animals were sacrificed at intervals up to 30 hours and the tissues were examined for radioactivity. Forty per cent of the injected activity left the site of injection in 15 minutes. The activity in the blood never exceeded 1% of the total amount injected. About 60% of the amount injected was excreted in the urine within 3 hours. The activity in the bile and intestine reached a maximal value of about 5% of the total in 2–3 hours. No activity could be detected in the expired air. The liver, muscles, bone, skin and other organs showed activities that were at no time more than a fraction of 1% of the total amount injected. These results were in contrast to previous work from these laboratories which showed that radioactive dicumarol is concentrated in the liver. Blood fractionation studies showed that the blood activity was associated with the erythrocytes, the white cells plus platelets and the albumin fraction. Similar results were obtained with cholecystonephrostomized dogs. Chromatographic studies of the urine from the injected mice revealed the presence of 1 radioactive product in fresh urine and 2 radioactive products in old urine or urine which had been heated with acid.

Method for separate collection of urine from kidneys of the trained dog. MERRILL P. SPENCER (introduced by J. MAXWELL LITTLE). *Dept. of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.*

This report describes a simplified procedure for the separate and simultaneous collection of urine from both kidneys of the dog. This method was developed for use with clearance techniques to study the differences between innervated and denervated kidneys of a trained animal over an extended period. Catheters made of non-irritating metal tubing, 1-mm. internal diameter and about 5 cm. in length are joined to a plastic tubing of the same diameter and 30 cm. in length. A 20-degree bend is made in the center of the metal tube and 6 holes drilled near the end opposite the plastic tubing. A guide is then made for each catheter from no. 4 ureteral catheters containing wire stilettes to produce rigidity. Female dogs of at least 12-kg. weight are used. After anesthesia each ureter is approached from a costo-vertebral angle. After incising it, the metal portion of the catheter is passed into the renal pelvis. The guide attached to the other end of the plastic tubing is then passed down the ureter, into the bladder, out the urethra, and to the exterior. With gentle traction on the guide, the plastic portion of the catheter will then follow. The guide is then detached leaving an artificial channel from the renal pelvis. The ureter is tied about the metal portion of the catheter and the incision closed.

The clear phase of superprecipitation reaction. S. S. SPICER. *Natl. Insts. of Health, U. S. Public Health Service, Bethesda, Md.*

The contractile precipitation of actin and myosin from colloidal suspension by adenosinetriphosphate (ATP) (Szent-Györgyi's superprecipitation) has been studied. Upon addition of ATP the turbid suspension forms a clear colloidal solution which subsequently yields to contractile precipitation. The clearing occurs at high and the precipitation and contraction occur at low ATP concentration. Factors enhancing the relative effectiveness of the ATP in causing clearing include high ionic strength, presence of Mg, or absence of Ca. Whereas Mg at pH 6.8 decreases 2 times the amount of ATP required to cause contraction, it reduces 20 times the nucleotide needed for clearing. In the presence of Mg the ATP requirement for clearing varies with pH having a maximum at about pH 6 and a minimum at pH 7, and increasing again from pH 7 to 8.5. This curve corresponds rather well to the pH curve of the ATP-ase activity of the solution. The two zones of high ATP requirement for clearing also correspond roughly with acid and alkaline zones of isoelectric precipitation of actin-free myosin. Inorganic pyrophosphate induces precipitation and contraction in an actomyosin solution rendered clear by ATP in the presence of Mg. This contraction coincides with the drop from pH 7 to 6 brought about by the formation of a complex between pyrophosphate and Mg. The pH for the maximum or minimum ATP requirement for clearing increases as the ionic strength decreases, indicating adsorption of anions by actomyosin. Anion adsorption is also indicated by the finding that both the upper and the lower limit of ionic strength where good contraction occurs increases as the pH decreases.

Role of the spinal cord in regulation of decerebrate spasticity in the cat. JAMES M. SPRAGUE (introduced by L. B. FLEXNER). *Dept. of Anatomy, Univ. of Pennsylvania, Philadelphia.*

Decerebrate spasticity can be restored to atonic, deafferented limbs by ipsilateral ablation of the medial cortex of anterior cerebellar lobe, by contralateral ablation of the fastigial nucleus, and

by transection of thoracic spinal cord. The cord effect, first demonstrated by Stella (1944), is shown by us to occur after ablation of anterior lobe of cerebellum and/or bilateral section of vestibular nerves. This restoration of spasticity to atonic, deafferented limbs is due to an intrinsic spinal mechanism. Its effect is not reproduced by section of the lumbosacral dorsal roots. It is identical with the Schiff-Sherrington effect described by Ruch (1936), (i.e. facilitation of foreleg extensors and inhibition of flexors in decerebrated cat, following postbrachial cord transection). Localization of the Schiff-Sherrington mechanism is revealed by marked ipsilateral extensor facilitation developed when decerebration is preceded by chronic hemisection at T 13. No change is present after hemisection in lower lumbar segments or after hindleg deafferentation. The cells involved are those described by Cooper and Sherrington ('40) in the lumbar ventral gray matter (L1–L6). This spinal mechanism is thus independent of other facilitating systems: stretch reflex, labyrinths and cerebellum. Its primary importance is further emphasized by its ability to restore decerebrate spasticity to forelegs previously rendered hypotonic by cerebellar ablation and destruction of Deiter's nuclei. It reveals itself of equal importance to the bulboreticular formation in the regulation of foreleg spasticity.

Squid rhodopsin. ROBERT C. C. ST. GEORGE,* JONAS M. GOLDSTONE* AND GEORGE WALD. *Biological Labs., Harvard Univ., Cambridge, Mass.*

The light-sensitive pigment of the squid retina, like vertebrate rhodopsin, has three absorption bands, α, β and γ, with maxima at 490, 365 and 279 mμ. Like vertebrate rhodopsin, it yields lumi-rhodopsin on irradiation; and this is converted in the dark to meta-rhodopsin, with an α-band at 492 mμ (ST. GEORGE AND WALD. *Biol. Bull.* 97: 248, 1949). Meta-rhodopsin is in equilibrium with a mixture of retinene$_1$ and the protein opsin. This equilibrium lies far over toward meta-rhodopsin below pH 6.5, and far over toward retinene and opsin above pH 10. Some basic group of opsin appears to be involved, possessing a pK about 8.5, suggesting an $—NH_2$ or —SH group. The equilibrium is displaced toward meta-rhodopsin also by cooling, showing that the formation of meta-rhodopsin from retinene and opsin is exothermic. The aldehyde group of retinene apparently is bound in meta-rhodopsin, since the latter is not attacked by hydroxylamine, a reagent which reacts almost instantly with retinene to form the aldoxime. In addition to the processes described, squid rhodopsin and its products decay irreversibly to retinene and protein. Below 15°C., this last reaction is almost negligible; but above 23°C. it goes rapidly. (This investigation was supported in part by the Medical Sciences Division of the Office of Naval Research.)

Effects of adrenal steroid compound F in depancreatized, cholesterol-fed cockerels. JEREMIAH STAMLER. *Cardiovascular Dept., Med. Research Inst., Michael Reese Hosp., Chicago, Ill.*

Pancreatectomy in chicks has no influence on blood glucose or lipid levels. When adrenal cortical extract is given to these birds, a marked acute hyperglycemic response occurs (*Proc. Soc. Exper. Biol. Med.* 75: 803, 1950); lipid levels are unaffected. The present study was undertaken to correlate these phenomena by an analysis of the acute and chronic effects of compound F in cholesterol-fed, depancreatized cockerels. Twenty 8-week-old depancreatized cockerels were divided into 2 groups, both fed a mash supplemented with ½% cholesterol and 5% cottonseed oil. One group received a daily injection of compound F (1–2 mg.). Unlike cortisone, this adrenal steroid was highly active in such small doses in chicks. It intensified cholesterol-induced lipemia (lactescence) and hypercholesterolemia (1502 *vs.* 692 mg.%), effected a fall in plasma Na (149 *vs.* 162 mEq/l.), and a marked retardation of growth (942 *vs.* 1824 gm. body weight at autopsy). Further, compound F induced a chronic insulin-resistant hyperglycemia (410 *vs.* 239 mg.%), with glycosuria in some birds. Hence, depancreatized chicks given compound F exhibit a steroid diabetes with significant alterations in cholesterol and lipid metabolism.

Relation of aortic pulse pressure curves both to left ventricular stroke volume and to output at every instant of systole. ISAAC STARR, T. G. SCHNABEL, JR.,* R. L. MAYOCK* AND J. H. AUSTIN. *Depts. of Therapeutic Research and Medicine, Univ. of Pennsylvania, Philadelphia.*

Simulating systole in cadavers at necropsy (*Circulation* 1: 1073, 1950) we secured simultaneous records of cardiac output, aortic arch and peripheral blood pressures at each instant. Such data, secured in 63 systoles in 7 cadavers in which systolic force, cardiac output, resistance and aortic elasticity varied widely, have now been analyzed. The contours of simultaneous aortic pressure and cardiac output curves are related; the latter has many features in common with the integral of the former, and an empirical base line correction improves our ability to estimate one from the other. $\int$ pdt from the beginning of the systolic rise to the dicrotic notch is related to total stroke volume by a family of curved regressions each corresponding to a different aortic elasticity and so to a pulse wave velocity. Our preliminary general formula is: *Total stroke volume = body surface area/1.63* [*77 log.* $\int$ *pdt* -2.73 *pulse wave velocity* -15.5], the units being cc., sq. m., mm. Hg sec., and m/sec., respectively.

The data needed to apply this formula in the clinic can readily be secured by the use of small plastic tubes (*Am. J. Physiol.* 159: 585, 1949) and Lilly manometers. The method's absolute accuracy is still under investigation. (This investigation is supported by a research grant from the National Heart Institute of the National Institutes of Health.)

Thrombocytopenic effect of normal plasma. MARIO STEFANINI AND JYOTI B. CHATTERJEA (introduced by JOSEPH H. PRATT). *Blood Research Lab., New England Center Hosp. and Dept. of Medicine, Tufts College Med. School, Boston, Mass.*

Normal citrated compatible human plasma was administered to 15 healthy individuals in approximate volume of 4 ml/kg. weight in adults and 6 ml/kg. weight in children, at average speed of 10 ml/minute. In 13, the platelet count dropped to 60–40% of the original value within 1 hour and returned to normal within 3–12 hours. Responsive subjects reacted equally to administration of plasma from different donors. No changes of the hemostatic mechanism were observed, no hemorrhagic manifestations appeared. Minor reactions occurred in 2 subjects (fever, urticaria) during the administration of plasma, but failed to influence the degree of thrombocytopenia induced. Microscopic observations of dry and wet preparations did not show clumping of platelets, but large, bizarre thrombocytes were ordinarily observed during the phase of recovery. The plasma administered appeared perfectly compatible by standard methods and, *in vitro*, recipient's serum failed to agglutinate or lyse the donor's platelets. When responsive subjects received: *a*) ordinary sodium citrate-dextrose solution (ACD); *b*) washed red blood cells; *c*) their own plasma, no drop in platelets occurred. Heparinization of recipient, passage of plasma through Seitz filters, storage of plasma at 4°C. for 21 days did not prevent the phenomenon. This, however, failed to occur when we administered plasma decalcified by passage through cation-exchange resin IRC-50 and subsequently treated with $Ca_3(PO_4)_2$ gel. Properties of the factor responsible for the thrombocytopenic effect of normal plasma are being investigated and will be reported in detail.

Effects of altitude and diet on the CO_2 content of flatus in man. F. R. STEGGERDA, W. C. CLARK* AND I. E. DANHOF.* *Dept. of Physiology, Univ. of Illinois, Urbana.*

It has been observed that when 300 cc. of room air are injected into the lower colon of man there is a progressive increase in the concentration of CO_2 as high as 40% in the collected flatus while going to simulated altitudes of 25,000 ft. More recently it has been noted that the collected flatus without dilution with room air may reach CO_2 concentrations of 80% within 10 minutes after reaching altitudes of 35,000 ft. Since these high concentrations of CO_2 are definitely above what could be explained on the basis of changes in diffusion equilibrium between body cells and the colon, other explanations for this gas must be sought. Experiments in progress indicate that the nature of the diet is a definite contributing factor. For example, low calorie—low residue diets show statistically significant differences in volume and CO_2 content of collected flatus compared with normal mixed unrestricted diets. By using O_2 administered into the stomach, as a reference gas, it was noted that the transit time for this gas to pass from the stomach to the lower colon was significantly more prolonged with the low calorie—low residue diet.

Effect of tobacco smoke on spontaneous activity of white rat. ARTHUR H. STEINHAUS, PAUL SCHULTZ* AND WILLIAM C. WILSON.* *George Williams College, Chicago, Ill.*

The spontaneous activity of white rats in freely revolving drums was determined in fresh air and in air filled at regular intervals during 12 hours of each day with cigarette smoke. The following conclusions appear warranted: *1*) Rats exposed to cigarette smoke regularly ran about 30% less than rats in fresh air. *2*) Rats exposed to smoke from cubebs (nicotine-free cigarettes) ran as much as rats in fresh air. *3*) When identical tobacco with varying amounts of added nicotine (0.2%, 0.5%, and 2.08%) was employed, the reduction of activity paralleled the nicotine content, the reduction being greatest with tobacco of highest nicotine content. *4*) Castrated male rats (observations limited to 2 rats) showed no difference in fresh or smoke-laden air. *5*) After injection of testosterone, castrated rats again reacted to smoke laden air as do normal males.

Experimental hypothalamic 'hyperphagia' and 'hypodipsia.' J. A. F. STEVENSON AND D. MONTEMURRO.* *Dept. of Physiology, Faculty of Medicine, Univ. of Western Ontario, London, Ontario, Canada.*

Observations of food and water intakes, urine output, body weight, and organ weights at autopsy, in a cat, followed for over a year after production of bilateral hypothalamic lesions, will be reported. The lesions, produced using a stereotaxic instrument, resulted in an increased food intake and obesity, as compared with the preoperative state and a control animal. However, hypothalamic 'hypodipsia,' which results from apparently similar lesions in the rat, was not present. Gonadal atrophy, which frequently follows such lesions in the rat, was observed in the cat. The histological localization of the hypothalamic lesions which produced 'hyperphagia'

in the cat and 'hyperphagia' and 'hypodipsia' in the rats will be described.

Blood proteins of Cryptochiton stelleri. DORIS STEWART,* WALTER DANDLIKER* AND ARTHUR W. MARTIN. *Depts. of Zoology and Biochemistry, Univ. of Washington, Seattle.*

Both the relative proportion of the various proteins and the total protein concentration in the blood of this animal may vary considerably from one individual to another. The range of total protein ordinarily found is from about 1–3%. The sedimentation diagrams of whole blood usually show three peaks which spread more rapidly than can be accounted for on the basis of diffusion. The fastest moving boundary spreads very rapidly and was not measured. At a protein concentration of 1.2% the values of $s_{20,w}$ for the slow and medium boundaries are roughly 16 and 49 S. Electrophoretically, three peaks are obtained from whole blood in phosphate buffer at pH 7.3 and $\Gamma/2$ 0.2. These comprise about 20, 60 and 20% of the total protein with mobilities of about -4, -5 and -8×10^{-5} cm.^{2} volt^{-1} sec.$^{-1}$ at a protein concentration of 0.7%. Areas and mobilities alike were measured on the descending boundary. Fractionation of the blood yields a blue protein material which gives one major sedimentation peak and corresponds to the medium-sized component in whole blood. Electrophoresis of this fraction showed two peaks, 85% and 15%. The former corresponds to the peak of mobility of -5×10^{-5}. The absorption spectrum at 1 atmosphere oxygen shows a detectable maximum at about 5500 Å. and a larger one at 3500 Å. This major component we believe to be hemocyanin, the roles of the other proteins are unknown. (Project supported by ONR)

'Veriloid' and cevadine on propulsive motility of the small intestine in dogs. J. CLIFFORD STICKNEY, DAVID W. NORTHRUP AND EDWARD J. VAN LIERE. *Dept. of Physiology, School of Medicine, West Virginia Univ., Morgantown.*

Propulsive motility of the small intestine was determined by giving 30 cc. of a 10% charcoal suspension in 10% gum acacia in water by stomach tube to adult dogs, unanesthetized and fasting. Five minutes later 5 μg/kg. 'Veriloid,' 20 μg/kg. cevadine or saline was given intravenously. Thirty minutes after intubation the dogs were sacrificed in order to determine the distance the charcoal mixture had traversed the intestine. In 10 dogs given 'Veriloid' 38.6% of the small intestine was traversed as compared with 71.5% in 11 controls (given saline). The difference: 32.9% is statistically significant at less than the 1% level. The percentage of the small intestine traversed in 10 dogs given cevadine was 68.3%, which is not significantly different from the control value.

A systematic error in infrared analysis for carbon dioxide in respiratory gas mixtures. RICHARD W. STOW (introduced by EDWARD J. BALDES). *Mayo Fndn., Univ. of Minnesota, Rochester.*

With the use of an infrared analyzer for determinations of CO_2 in concentrations of about 5 vol.% in mixtures containing various proportions of O_2 and N_2, a systematic error of around 0.5 vol.% has been encountered. The basis of the analysis is a measurement of the infrared absorption by carbon dioxide present in the sample. A radiation detector is employed which is sensitive only in the spectral regions where CO_2 absorbs. It is similar in principle to the detector used in the Luft analyzer. The absorption cell for use in analysis of respiratory gas has a thickness of 0.5 mm. and a volume of 0.1 ml. This systematic error is due primarily to differences in the intensity of infrared absorption by CO_2, depending on the proportions of other gases present in the sample. The error is larger at a total gas pressure of 0.1 atmosphere than at 1 atmosphere. It is decreased, but not eliminated, by dilution of the sample with a nonabsorbing gas. When cells of 0.12–8 mm. thickness are used, the error increases from 0.4–0.8 vol.%. (Work carried out in the Section of Biophysics and Biophysical Research, Mayo Clinic and Mayo Foundation, under the direction of Dr. Edward J. Baldes.)

Protection of mice against irradiation by dog plasma and spleen perfusates. AGNES N. STROUD* AND AUSTIN M. BRUES. *Div. of Biological and Medical Research, Argonne National Lab., Chicago, Ill.*

Dog plasma, administered intravenously in maximal doses (0.5 ml. diluted with 0.5 ml. White's solution) protects mice partially against 800 r x-ray when given immediately before irradiation. This material is somewhat toxic to mice. By incubating the blood from which the plasma is obtained or by perfusing it through the spleen of the same dog for 5–6 hours, it loses its toxicity but retains its protective potency. Intraperitoneal administration under the same conditions is ineffective, as is intravenous injection immediately following irradiation. Although the amount of plasma is sufficient to cause a great increase in plasma volume during irradiation, corresponding dosages of beef plasma albumin have no effect. White's medium perfused through the normal dog spleen is inactive. If the spleen has been lead-protected while the dog was irradiated with 450 r x-ray, however, perfusion of this organ with White's medium yields a very active perfusate. This perfusate is likewise effective only when injected before irradiation.

Changes in resistance to pulmonary blood flow due to altering the inspired gas tensions. R. C.

STROUD* AND H. RAHN. *Dept. of Physiology and Vital Economics, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Pulmonary resistance was calculated as the pressure drop between the pulmonary artery and vein divided by the rate of blood flow. Measurements were obtained on nembutalized (28 mg/kg.) dogs with catheters in the main pulmonary artery and vein. Pressures were determined by means of saline manometers. Blood flow was measured by the Fick method. Control determinations were made while the dog breathed room air. In none of the dogs did the breathing of the test gas mixtures cause a significant change in the pulmonary venous pressure. Five dogs showed no change in resistance while for 22 dogs the calculated resistance increased 25 and 48%, respectively, breathing 8 and 15% O_2, and decreased 11% with 30% O_2. Five dogs breathing 5% CO_2-25% O_2-70% N_2 showed no significant resistance change. Three dogs which showed an increased resistance with low oxygen were sympathectomized from the middle cervical ganglia through T-9. Three weeks after the operation all failed to show an increase in resistance with low oxygen. The lack of resistance change in some of the dogs breathing low oxygen may be due to the depth of anesthesia. In unanesthetized dogs with indwelling tubes in the pulmonary artery it was found that the pulmonary arterial pressure increased about 100% when 8% oxygen was breathed. One of these dogs had previously failed to show this response under Nembutal anesthesia.

Blood and urinary sugar levels after intravenous infusion of glucose, fructose and invert sugar in human subjects. IRWIN STRUB,* W. R. BEST,* EVELYN MAREK* AND M. I. GROSSMAN. *U. S. Army Med. Nutrition Lab., Chicago, Ill.*

Nine normal adult male fasting subjects each received, on separate days, intravenous infusions of 10% solutions of glucose, fructose and invert sugar. The rate of infusion was 1.5 liters in 3 hours for glucose and 3 liters in 3 hours for fructose and invert sugar. The highest total blood sugar levels were 187.4 ± 9.62 (S.E.) mg.% for the glucose infusion, 190.7 ± 8.83 for fructose and 242.0 ± 21.12 for invert sugar. The highest blood fructose levels were 92.0 ± 10.32 mg.% for the fructose infusion and 51.1 ± 5.37 for invert sugar. The percentage losses of infused sugar in the urine over a 5-hour period were 1.52 ± 0.46 for glucose, 6.81 ± 0.28 for fructose and 5.90 ± 0.73 for invert sugar. The mean percentages of urinary sugar excreted as fructose were 87.7 for the fructose infusion and 49.9 for invert sugar. The volumes of urine for the 5-hour period after beginning the infusion were 1493 ± 35 for glucose, 2290 ± 296 for fructose and 2475 ± 598 for invert sugar. It is concluded *a*) that under the conditions of this study invert sugar is not utilized at twice the rate of glucose, in contrast to the findings of Weinstein (*Med. Ann. Dist. Columbia* 20: 186, 1951) who infused smaller total amounts of sugar, and *b*) that fructose and invert sugar show approximately the same percentage urinary loss.

Effect of experimental hypothyroidism upon gastric secretion in the rat. DAVID SUN,* HARRY SHAY, HERMAN SIPLET* AND MARGOT GRUENSTEIN.* *Fels Research Inst., Temple Univ. School of Medicine, Philadelphia, Pa.*

Using the rat preparation developed in our laboratory (*Gastroenterology* 5: 43, 1945) we studied the effects of experimental hypothyroidism upon gastric secretion. Animals weighing 90 to 1100 gm. were subjected to thyroparathyroidectomy and mock thyroparathyroidectomy. Six to 8 weeks later after proper starvation the pylorus was ligated, the stomach lavaged with saline and emptied completely. Four hours later, the animal was sacrificed and the gastric contents were collected quantitatively. These were measured and analyzed for free and total acidity, pH, peptic concentration and activity when enough material was available. Blood samples were collected and analyzed for cholesterol, calcium and phosphorus. In some cases, we had to pool blood specimens to permit all of these determinations. Volume of secretion and output of total acid per 100 gm. body weight were calculated. The whole tissue mass in the region of the thyroid was subjected to serial section and examined microscopically for thyroid fragments. Results showed that there was a definite decrease in the volume and output of total acid in the completely thyroparathyroidectomized animals as compared with those of the mock thyroparathyroidectomized and unoperated controls. These animals in which small fragments of thyroid were found showed no significant change in volume and output of total acid. In another group of animals radioactive iodine was injected intraperitoneally in an effort to destroy the thyroid tissue only. In a third group the effect of thiouracil was investigated. The results will be compared with those of thyroparathyroidectomized and control animals.

Enlargement of the liver following whole body x-irradiation. HELEN SUPPLEE AND CECIL ENTENMAN (introduced by JAMES M. D. OLMSTED). *Biochemistry Branch, Biological and Med. Sciences Div., U. S. Naval Radiological Defense Lab., San Francisco, Calif.*

A marked increase in liver size has been observed in adult female Sprague-Dawley rats subjected to large doses of total body x-irradiation. With doses ranging from 400–500 r the liver weights at 48 hours postirradiation were 18–60% above their

respective fasted unirradiated controls. The liver enlargement is not due to edema or to the excessive accumulation of lipids. The increase in liver mass occurred rapidly following irradiation, a significant increase being noted as early as 6 hours following a dose as low as 550 r. At all doses studied the maximum increase in liver mass had occurred by approximately 24 hours postirradiation. At a total dose of 1200 r given at dose rates of 6.8, 24.6, 82, and 210 r/minute, no significant differences in the extent of enlargement were noted. Adrenalectomy performed immediately before irradiation with 1200 r did not prevent liver enlargement. At the same total dose cysteine (950 mg/kg.) injected intravenously just before irradiation was also ineffective. Liver enlargement was noted in young and old rats, both male and female.

Effect of acute changes in glomerular filtration rate on water and electrolyte excretion. A. SURTSHIN, C. B. MUELLER* AND H. L. WHITE. *Depts. of Physiology and Surgery, Washington Univ., St. Louis, Mo.*

Unanesthetized dogs with explanted ureters and one kidney chronically denervated have equal urinary excretions of water, sodium, potassium, inulin and PAH from the two kidneys during water diuresis. The PAH and inulin clearances are unchanged from those existing before denervation. There is no preferential excretion of injected sodium chloride by either normal or denervated kidney. With pain, some animals show greater fall in PAH and inulin clearances in the normal than in the denervated kidney. Deep ether anesthesia and, to a lesser extent, deep pentobarbital anesthesia produce fall in PAH and inulin clearances in the normal kidney, with less or no fall in the denervated. Acute falls in inulin clearance are accompanied by greater homolateral falls in sodium and water excretions. There is no detectable renal sympathetic tone in normal, resting unanesthetized dogs.

Weight and water comsumption of X-irradiated rats. E. SUTTON* AND L. F. NIMS. *Biology Dept., Brookhaven Natl. Lab., Upton, L. I., N. Y.*

Rats fed *ad libitum* after x-irradiation lose weight for a period of time proportional to the dose of x-rays received. The time required to regain the weight lost since the day of irradiation is a linear function of the dose to about 600 r, and the effects of dose increments as small as 50 r can be detected. Fasted irradiated rats lose weight after the first day or so slightly faster than do fasted controls. The water consumption of irradiated rats is greater during the first 24 hours than in control rats. A second period of increased water consumption occurs in fed irradiated rats which is apparently associated with recovery. (Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

Manual artificial respiration in rats and guinea pigs. HENRY E. SWANN, JR.,* PETER V. KARPOVICH AND DAVID B. DILL. *Chemical Corps Med. Labs., Army Chemical Center, Md., and Springfield College, Springfield, Mass.*

Manual artificial respiration was studied in rats and guinea pigs after immersion in fresh water. Survival from drowning was not affected by manual artificial respiration. Possible causes of this ineffectiveness were ventricular fibrillation, hemolysis and associated plasma potassium changes, rigor mortis of thoracic muscles, and obstruction of the air-ways. No fibrillation was found in animals even 8 minutes after beginning of submersion. Hemolysis was slight, hemoglobin in plasma ranging from 5 to 22 mg.% in rats bled while nembutalized and from 24 to 131 mg.% in rats bled after drowning. Corresponding plasma potassium ranges were 4.5 to 8 mEq/l. in controls and 5.5 to 9 mEq/l. in others. These slight changes cannot account for death of drowned rats. The lungs and trachea of drowned rats and guinea pigs were filled with froth and fluid. Manual artificial respiration failed to move air into or out of drowned animal lungs. Animals, in which respiration was stopped by trauma, had adequate pulmonary ventilation initially. This decreased by nine-tenths within 2 minutes and was zero at the end of an hour. Thoracic ventilation was large and remained undiminished for 22 hours. Thus, rigor mortis of thoracic muscles is not a cause of the ineffectiveness. It is concluded that the ineffectiveness of artificial respiration in reviving rats and guinea pigs from drowning depends on obstruction of air-ways with froth and fluid.

Distribution of fixed carbon. ROBERT W. SWICK, DONALD L. BUCHANAN AND AKIRA NAKAO (introduced by THEODORE N. TAHMISIAN). *Div. of Biological and Med. Research, Argonne Natl. Lab., Chicago, Ill.*

Rats and mice on stock diets were continuously exposed to air containing a constant low partial pressure of $C^{14}O_2$. This maintained an unchanging specific activity (s.a.) within the CO_2-bicarbonate pool of the animal. Within 2 weeks the radioactivity of the total liver carbon reached a constant level. This constant s.a. was also found in the livers of mice born and raised in this labeled atmosphere. Such agreement was evidence that the total fixed carbon of the liver had become isotopically saturated. Therefore, a survey of the distribution of C^{14} in this tissue should quantitatively mirror the true distribution of CO_2-derived carbon in the natural state. Approximately 3% of the total liver carbon was derived from CO_2. About 70% of the fixed carbon was

found in protein, 12% in carbohydrate, 5% in lipids and 1% in a crude nucleic acid fraction. Only 4% of glycogen carbon and 1.4% of glycerol carbon represented fixed carbon. About 1% of the carbon in the unsaponifiable lipids and much less in the fatty acids was derived from CO_2. Nearly 80% of the fixed carbon in protein was in α-carboxyl groups of amino acids. While 73% of the guanidino carbon of arginine was derived from CO_2, the ornithine moiety was practically devoid of fixed carbon. Other essential amino acids examined and tyrosine contained essentially no fixed carbon. As measured by these experiments the fixed carbon distribution differs considerably from that reported after isotope exposures of shorter duration.

Pretreatment with cysteine in partial body x-irradiation. MARGUERITE N. SWIFT, S. TOM TAKETA AND VICTOR P. BOND (introduced by J. S. THATCHER). *U. S. Naval Radiological Defense Lab., San Francisco, Calif.*

The effect of cysteine on morbidity and mortality in each of two clinically distinguishable radiation syndromes, resulting from exposure of two mutually exclusive portions of the body, has been determined. The x-ray mortality curves obtained for rats irradiated with the abdomen only exposed indicate approximate LD_{50} values of 1400 r for cysteine-pretreated animals (950 mg/kg. i.v.) and 960 r for controls injected with equivalent volumes of 5% NaCl. The syndrome typical of abdominal irradiation in the rat (diarrhea, moderate weight loss, 4–5 day mean survival time) was observed in both groups. Among animals irradiated with the abdomen shielded, the x-ray LD_{50} values obtained were approximately 3010 r for cysteine-pretreated rats and 1700 r for controls. Both treated and control animals exhibited the exudative oral lesions, severe weight loss, absence of diarrhea and 11–12 day mean survival time characteristic of this type of partial body irradiation. In cysteine-pretreated animals, with either type of shielding, the severity of illness was identical to that seen in control groups exhibiting the same percentage mortality. The greater efficiency of cysteine protection in abdomen-shielded rats was even more strikingly demonstrated in another series of experiments in which x-ray dosage was held constant and cysteine dosage varied. The estimated x-ray LD_{100} levels were used for both abdomen-exposed and abdomen-shielded animals; 1150 r and 1850 r respectively. Under these conditions, 50% survival was obtained with 400 mg. cysteine/kg. in abdomen-exposed rats, and with 50 mg/kg. in abdomen-shielded rats.

Effect of vitamin B complex deficiency and of amino acid deficiency on chick liver ascorbic acid. JOSEL SZEPSENWOL, PHYLLIS L. STROBEL* AND MARY HANSON PARTRIDGE.* *Depts. of Anatomy and Biochemistry, Emory Univ. School of Medicine, Emory University, Ga.*

Four groups of 6-week-old chicks were maintained for 4–6 weeks on diets deficient respectively in: *1*) the vitamin B complex; *2*) thiamine; *3*) niacin; *4*) essential amino acids. Basal ration no. 240 of Kline *et al.* (*J. Biol. Chem.* 99: 295, 1932) was employed and the B vitamins were destroyed by 20–24 hours of autoclaving. Casein was omitted from the amino acid deficient diet, in which corn and middlings provided the dietary protein. For normal controls, chicks of the same age were maintained on the basal ration adequately supplemented with B vitamins. Each group consisted of 16–24 animals. At the end of the experimental period, the chicks were sacrificed and the livers analyzed individually for ascorbic acid by the method of Roe and Kuether (*J. Biol. Chem.* 147: 399, 1943). The mean ascorbic acid values for each group, in mg/100 gm. fresh liver, were as follows: controls, 38; B complex deficiency, 25; thiamine deficiency, 24; niacin deficiency 25; amino acid deficiency, 27. These results indicate that various states of nutritional deficiency impair the capacity of the chick either to synthesize ascorbic acid, or to store it in the liver. This is in agreement with similar findings reported in the literature for vitamin A, thiamine or riboflavin deficiency in the rat.

Some properties of actomyosin strips of heart muscle. MAX TAESCHLER* AND R. J. BING. *Dept. of Medicine and Physiology, Univ. of Alabama Med. School, Birmingham, Ala.*

Strips of heart muscle of 2 mm. diameter and 3–4 cm. in length were dissected from the ventricular muscle of dogs. The muscle was washed for 8 days in 50% glycerol according to Szent-Gyorgyi. From this preparation, muscle fibers with a diameter of from .4 to .7 mm. diameter and a length of 2 cm. were dissected. The muscle was suspended in an isotonic solution with a cation composition identical to that of heart muscle. Isotonic and isometric contractions were recorded following the addition of .8% of the Na salt of ATP. On isometric contraction, the tension developed, as well as the speed of contraction, was less than that of actomyosin preparation of rabbit psoas. It is probable that this is the result of specific qualities of heart muscle. When the preparation was loaded and its contraction recorded isotonically, the degree of contraction and work performed were proportional to the logarithm of time. Augmenting the load resulted in an increased initial length and in a greater output of work. However, the speed and amplitude of contraction were decreased. After a certain load was exceeded, the work declined. The results indicate similari-

ties between the properties of the intact heart and the actomyosin preparation of heart muscle.

Comparative uptake of carrier and carrier-free Ca^{45} in rats. R. V. TALMADGE, F. W. KRAINTZ, L. K. WANG AND L. KRAINTZ (introduced by H. E. HOFF). *Rice Institute, Houston, Tex.*

Until recently, the Ca^{45} available for biological studies contained additional stable calcium. Since calcium in small amounts has marked effects on mammalian physiology, the possible influence of the carrier accompanying the radio-isotope must be considered. This work was undertaken for the purpose of studying the uptake, distribution, and disappearance of Ca^{45} in male rats. *Group I* received less than 0.25 mg. of calcium/100 gm. of body weight (physiologically considered carrier-free), while *group II* received app. 5 mg. for the same unit weight. Both groups received equal amounts of radiocalcium. The rats in *group I* showed a rapid uptake by bone and equally fast disappearance from blood, while the activity (% injected dose/gm. of tissue) in those soft tissues studied, approached but never surpassed that of blood. The rats in *group II* showed a much slower uptake by bone, and a corresponding lag in disappearance from the blood. In contrast with *group I*, muscle activity was consistently lower than that of blood, but the thyroid, liver and prostate showed activities up to four times that of blood for as long as 24 hours after injection. The seminal vesicles reached a value of 25% of bone and ten times that of blood activity. While the deposition of Ca^{45} in the seminal vesicles gives rise to interesting interpretations, the main emphasis of this study is concerned with the differences in the activities of the tissues studied following administration of carrier and carrier-free Ca^{45}. (Aided in part by a grant from the Atomic Energy Commission.)

Radiation area of the human body. CRAIG L. TAYLOR AND ARMAND GUIBERT.* *Dept. of Engineering, Univ. of California, Los Angeles.*

A method for determining directly the area of that part of the body surface which takes part in radiation exchange with the surround is described, together with results obtained for 3 subjects of divergent body shape and for 4 different body postures. The analytical basis of the method is developed from the concept of the local shape factor integrated over all space, permitting, by use of the reciprocity theorem, an evaluation of the equivalent total projected area, defined as the radiation area. The experimental procedure involves the photographing of the human subject at a fixed distance in excess of 15 ft., from 32 viewing angles chosen to sample effectively the surface of a hemisphere described about the sagittal plane as equator. The planimetered areas from each photograph, representing projections of the body, are plotted as functions of 2 angles which define the viewing position, and by a graphical integration procedure the total area 'seen' by the whole of the surround is obtained. Data were obtained by this method for the nude radiation areas of 3 subjects, of heavy, medium and light body weights. The postures were erect, semi-erect, seated and crouched. The medium subject was measured also with clothing. While radiation area is a function of posture, as expected, no evidence for a relationship with body type was found. The results are best expressed in the form of a factor expressing radiation area as a proportion of the total body surface area as given by the Dubois formula. The average values of this radiation area factor for nude subjects in the 4 postures from erect to crouched are 0.77, 0.72, 0.70 and 0.65, respectively. The average ratio of clothed projected area to nude projected area was found to be 1.14. This offers a short method for obtaining clothed radiation areas.

Basal cardiac output and body composition in developing obesity in man. HENRY LONGSTREET TAYLOR, WALTER CARLSON,* JOSEF BROZEK AND ANCEL KEYS. *Lab. of Physiological Hygiene, Univ. of Minnesota, Minneapolis, and Mental Health Research Lab., State of Minnesota, Hastings.*

An attempt was made to induce excess consumption of calories for 6 months in 7 healthy male schizophrenics age 33–45 years. Body fat was estimated from immersion densitometry with correction for residual air in the lungs during weighing under water (nitrogen washout method). Satisfactory cardiac outputs in duplicate by the acetylene method, corrected to direct Fick levels, were obtained in all 7 men. Four of these gained on the average 18.2 kg. (range from 13–21 kg.), while the other 3 gained 3.9 kg. (range 2–5 kg. of body weight). The former group added 13.3 kg. of fat and 4.9 kg. of fat-free tissue while the latter added 3.1 kg. of fat and 0.8 kg. of fat-free tissue. Changes in the blood pressure and pulse rate of both groups were minimal. The cardiac output in the obese group increased from 4.36 at the beginning to 4.87 l/minute at the end of the overnutrition period while that of the group making small weight gains had values of 5.10 and 4.88 l/minute, respectively. The cardiac output/kg. of fat-free tissue in the weight gaining group was 73.3 cc/minute before and 75.64 after the fattening period with no change in the A-V oxygen difference. Similar figures for the second group were 84.8 and 80.1 cc. The estimated circulation in the added fat-free tissue in the weight gaining group was 103.6 cc/kg/minute. The additional fat-free tissue which was added by the obese group appears to account for the increased basal cardiac output.

Nomogram for cardiac synergism of calcium, potassium and digitalis bodies (for mammalian myocardium). RICHARD M. TAYLOR* AND WILLIAM T. SALTER. *Labs. of Pharmacology and Toxicology, Yale Univ. School of Medicine, New Haven, Conn.*

A nomogram for contractility involving calcium, potassium and digitalis-like drugs has been presented (*Federation Proc.* 9, 1951) for the frog heart at 26°C. A companion nomogram has now been prepared for mammalian myocardium, i.e. the papillary muscle of the cat, at 38°C. On comparison, the two nomograms show certain differences. Chief among these is the relative insensitivity of the mammalian myocardium to changes in potassium concentration within a broad physiological range. The sharp reversal in response at 0.0048 N potassium (characteristic of the frog heart at 26°C.) is absent with the mammalian myocardium at 38°C. Consequently, within a broad physiological range, the traditional calcium : potassium ratio has no meaning for the force of myocardial contraction. At very low and very high concentrations of potassium disturbances in conduction and contractility do occur; but the potassium concentrations involved are artificial, or at least toxicological.

Mechanism normally suppressing monosynaptic reflex discharge of spinal motoneurones in man. ROBERT D. TEASDALL,* HELEN W. LANGUTH* AND J. W. MAGLADERY. *Subdept. of Neurological Medicine, Johns Hopkins and Baltimore City Hospitals, Baltimore, Md.*

Action potentials of low-threshold H reflexes are readily recorded from calf and large flexor and extensor muscles of thigh following submaximal shocks to the appropriate nerves of supply. Regardless of 'clinical' reflex activity or tone, they have never been detected in 20 normal persons nor in 13 patients with upper motor neurone lesions above middle of pons, from small muscles of hand, foot, or anterolateral region (*Bull. Johns Hopkins Hosp.* 88: 499, 1951, and *Federation Proc.*, March 1952). When, however, those muscle groups were studied in 25 patients with upper motor neurone signs due to lower brain stem or spinal lesions proximal to the tested spinal segments, monosynaptic H reflexes were always detected, and occasionally C deflections were reflexly evoked by passive muscle stretch. Release of these low-threshold myotatic reflexes from suppression occurred in absence of conscious sensory deficit, ataxia, or 'cerebellar' signs. Apparently, in man, some mechanism controlling motoneurone excitability extends up to lower pontine levels by unidentified pathways. If, as seems possible by analogy with animal studies, these paths are through cerebellum, their interruption does not produce gross clinical signs commonly attributed to cerebellar lesions. Release of motoneurones from this suppression does not appear requisite for spasticity as encountered by the clinician.

Acetylcholine on heart rate in unanesthetized compared with anesthetized dogs. HARRY A. TEITELBAUM* AND W. HORSLEY GANTT. *Pavlovian Lab., Phipps Psychiatric Clinic, Johns Hopkins Univ., Baltimore, Md.*

Dogs were injected by a method previously reported (H. A. TEITELBAUM AND W. H. GANTT. *Tr. Am. Neurol. Assoc.* p. 72, 1948; OWENS AND GANTT. *Am. J. Physiol.* 163: 740, 1950) for the isolation of the experimental animal from the experimeter, as the presence of the experimenter has a marked effect on the heart rate. Acetylcholine chloride 1% solution .02 cc/kg. was injected intravenously. Both groups of dogs were injected from a distance, shielded from the presence of the experimenter as well as from external stimuli. The duration of injection was about 3 seconds. In the intact *unanesthetized* dogs acceleration of heart rate almost completely replaced inhibition following ACh; the heart rate (HR) increased from a control of 80–100 to about 150, reaching a peak in 15–20 seconds and returning to normal in about 60 seconds. In the *anesthetized* animal complete inhibition of the HR lasting about 3 seconds appeared in 15–20 seconds after injection; this was succeeded by acceleration attaining a maximum in about 25 seconds and returning to normal in about 60 seconds. The control HR in the anesthetized dogs was higher (100–150) as the result of the anesthetic (Nembutal 0.5 mg/kg.); the peak HR due to ACh was 175–250. The chief difference in the 2 groups of animals was the absence of th inhibitory effect on the HR in the intact dog. The method of injecting drugs from a distance enables one to study the effect of drugs in the intact animal uncomplicated by extraneous stimuli, especially the presence of a person.

Demonstration of anti-ketogenic effect of insulin in surviving liver slices obtained from normal rats. JAY TEPPERMAN, HELEN M. TEPPERMAN* AND JANET M. DEWITT.* *Dept. of Pharmacology, State Univ. of New York College of Medicine, Syracuse, N. Y.*

Shipley (*Am. J. Physiol.* 144: 51, 1945) reported that the *in vitro* addition of insulin failed to inhibit ketogenesis by rat liver slices. In a few similar experiments in which Lilly (HGF and phenol-free) crystalline insulin was used in a concentration of 1 unit/ml. of Krebs-Henseleit phosphate buffer no consistent effect of the insulin on ketogenesis was demonstrated. Therefore, the following experiment was used: rats were anesthetized with pentobarbital sodium and, after suitable

ligatures were in place, two lobes of liver were removed and stored in a moist, chilled chamber. An injection of 3 U of insulin in 0.25 ml. of saline was then made into the portal vein over a period of about 1 minute. Three minutes after the end of the injection the remaining lobes of liver were removed. Slices prepared from both pre- and postinjection liver samples were incubated in insulin-free phosphate buffer at pH 7.4 for 2 hours and ketone body production was measured as acetone. Slices prepared from the post-injection blocks consistently made fewer ketone bodies than did their pre-injection controls.

Effect of certain pituitary growth hormone preparations on ketogenesis by liver slices in vitro. JAY TEPPERMAN, HELEN M. TEPPERMAN,* AND JANET M. DEWITT.* *Dept. of Pharmacology, New York State College of Medicine, Syracuse.*

It was recently reported (TEPPERMAN AND TEPPERMAN. *Ann. New York Acad. Sc.* In press) that enhancement of ketogenesis by surviving liver slices occurred on the addition to the medium of 1 mg/ml. of either Armour 22KR1 or Armour J21609R purified pituitary growth hormone (GH). The percentage increase over the control level was calculated for each rat from a comparison of the mean activity of 4 slices with hormone and 4 without. Heat inactivated J21609R and crystalline bovine plasma albumin were without effect. These observations have been extended to include 20 experiments in which the mean increase in ketogenesis was about 20% with a range of 10–36% in individual experiments. It is noteworthy that these results were obtained with 300–400-gm. rats, and that a few experiments done on rapidly growing 200-gm. rats yielded negative results. Similar experiments were done on large rats with a GH preparation made by A. E. Wilhelmi and another (Somatotrofin) prepared by Horner, Ltd. In 9 experiments done with each of these preparations no stimulation of ketogenesis was observed, although Armour J21609R yielded a substantial increase when tested on some of the same livers. All 3 preparations were prepared according to Wilhelmi's method and all 3 are reputed to show good GH potency.

Effects of cerebral lesions on intellectual functioning in man. H. L. TEUBER,* W. S. BATTERSBY* AND M. B. BENDER. *Psychophysiological Lab., New York Univ. College of Medicine, New York City.*

Removal of frontal lobe tissue, for neoplasm, has been reported to result in intellectual deficits more severe than those seen after comparable lesions in other cerebral areas (Rylander, Halstead). These authors concluded that the integrity of the frontal lobes is more important for intellectual functioning than the integrity of other parts of the brain. The present study tested the validity of this generalization by surveying intellectual functions (over a period of several years) in veterans with gunshot wounds of the brain sustained 5–7 years before initiation of testing. Special studies were made of 2 subgroups: a first of 40 men had penetration through the frontal region (anterior group); another group of 40 had penetration through the posterior parieto-temporal, parieto-occipital, or occipital regions (posterior group). Performance of both groups was contrasted with that of 60 veterans with peripheral nerve injuries sustained in combat but without head injury (controls). Procedures included complex sorting tests, tests of ability to discover hidden figures, and tests of problem solving ('practical reasoning'). Results (obtained in individual sessions) indicated some deficits in both brain injured groups, as compared with the controls. However, deficits in the posterior group equalled or surpassed those found in the group with anterior lesions. Thus, gunshot wounds of the frontal lobes failed to produce significantly greater deficits than injuries in the posterior lobe substance, in contrast to the observations by Rylander and Halstead in cases of cerebral neoplasm (slides). (Work done under contract V 1001M-176 with the U. S. Veterans Administration.)

Fatigue of the sweat glands. JØRN HESS THAYSEN, IRVING L. SCHWARTZ AND VINCENT P. DOLE (introduced by DAVID P. C. LLOYD). *Hosp. of Rockefeller Inst. for Med. Research, New York City.*

In conjunction with the method for quantitative collection of sweat (*Proc. Soc. Exper. Biol. & Med.* 77: 412, 1951), a technique has been developed for the enumeration of functioning sweat glands. Iodine is diffused into bond paper by sublimation; it reacts with the starch in the paper to form a perceptible dot when as little as 10^{-4} mg. of water is absorbed. Prints of the glands thus may be taken consecutively without preparation of the skin. Repeated intradermal injections of mecholyl (4 mg.) or a steady intradermal infusion (0.125 mg/min.) evoked decreasing outputs of sweat per gland until a refractory state was reached. Recovery required 1 to 6 hours, depending on the degree of exhaustion. Reflex sweating, produced by environmental temperature of 40–45°C., decreased during 4 hours of observation. That the decrease was due to fatigue of the peripheral unit rather than to diminished stimulation was suggested by the following observations: *1*) rectal temperatures rose 0.2–0.3°C. and remained elevated, or increased further during an experiment; *2*) the output per gland diminished while the number of functioning glands remained maximal; *3*) the frequency of excretion waves, reflecting

periodic nervous stimulation, was unchanged; 4) infiltration of novocaine, sufficient to block reflex sweating for 20–40 minutes, was followed by hypersecretion as compared to a symmetrical area that had continued to function; 5) the output of sweat following intradermal injection of mecholyl (4 mg.) was diminished by preliminary reflex sweating. These results suggest that sweating, even within moderate physiological limits, produces fatigue of the excretory units.

Significance of areas of low ESR. PRICE E. THOMAS* AND IRVIN M. KORR. *Kirksville College of Osteopathy and Surgery, Kirksville, Mo.*

These studies are concerned with the functional significance of, and the physical basis for the areas of low electrical skin resistance (ESR) found on the trunks of human subjects. The patterns of segmental distribution vary with the subject, but may be constant over many months in a given subject. The low-resistance areas are frequently associated with other regional or segmental disturbances (*Federation Proc.* 7–10). The explorations are conducted under nonsweating conditions, eliminating frank moisture as a factor in ESR. Gravimetric measurements of insensible perspiration also showed no differences between symmetrical low-R and high-R areas. Colorimetric studies of thermo-regulatory sweating, however, revealed patterns of regional or dermatomal differences in the sequence of initiation of sweating which often resembled the subject's ESR pattern, the low-R areas sweating first. Similarly, studies with the spot-check method of Randall, under conditions of our ESR explorations, showed that the low-R areas had higher active-gland counts during the infrequent spontaneous bursts of activity than the high-R areas. ESR fell sharply during even slight activity peaks, but the low-R areas remained distinguishable during the long inactive periods. The relation of resting ESR to sweat-gland potentials is now being investigated. Low ESR appears, therefore, to reflect facilitated local sudomotor pathways; the sweat glands in such areas are in an altered physiological state electrically detectible in the absence of sweating. (Supported by research grants from National Institutes of Health and American Osteopathic Association; Contract Nonr-243(00) with Office of Naval Research.)

Urine flow and solute excretion during osmotic diuresis. DAVID D. THOMPSON AND MARTHA J. BARRETT (introduced by DAYTON J. EDWARDS). *Dept. of Physiology, Cornell Univ. Med. College, New York City.*

It has been demonstrated by Rapoport *et al.* that urine flow in hydropenia is determined by the quantity of osmotically active solute presented for excretion, independent of the nature of the loading solute. The urinary load was varied in their experiments by varying the rate of infusion and hence the plasma concentration of the loading solute. Urinary load may also be varied by varying the rate of glomerular filtration, keeping the plasma concentration of the loading solute constant. We are presently engaged in a comparison of the urine flow-solute load relationship in the dog under these two experimental circumstances, utilizing mannitol as the loading solute. Glomerular filtration rate is varied by controlled inflation of a balloon catheter positioned in the aorta above the origins of the renal arteries. To date nine experiments have been made.

Mechanisms responsible for acute toxicity of a phosphate glass. C. S. TIBDALL, R. E. GOSSELIN AND R. M. RENE (introduced by E. MAYNARD). *Div. of Pharmacology and Toxicology, Dept. of Radiation Biology, School of Medicine and Dentistry, Univ. of Rochester, Rochester, N. Y.*

The enzymatic hydrolysis of commercial hexametaphosphate (HMP) injected into rats produced the equivalent of 0.62 mols H^+ per mol of phosphate released. The rapidity of this hydrolysis created a severe systemic acidosis within an hour after parenteral administration of Na-HMP. At sublethal doses most of this acid appeared in the urine within 24 hours. The calcium salt of HMP, being more slowly hydrolyzed, caused less disturbance of body buffers, and was less toxic than Na-HMP. Hexametaphosphate and its hydrolysis product, orthophosphate, also depressed the extracellular level of ionized calcium. The latter was demonstrated by prolongation of the *Q-T* interval in the EKG during intravenous infusions of Na-HMP. Although this hypocalcemia is thought to have been responsible for cardiac arrest under certain circumstances, acidosis was probably the usual cause of death from HMP.

'Seizures' in a peripheral nerve preparation with feedback. J. E. P. TOMAN AND M. E. TOMAN.* *Dept. of Pharmacology, Abbott Labs., North Chicago, and Psychosomatic Inst., Michael Reese Hosp., Chicago, Ill.*

When action potentials of the frog sciatic nerve are amplified and fed back to stimulate the opposite end of the nerve trunk, a simple reverberating circuit is made possible for analogy with the behavior of more complex central nerve nets. Depending upon the relation between feedback gain and fiber excitability distribution, alternative states of activity can be found which satisfy the stability conditions *a*) that output equals input, and *b*) that output/input slope is less than 1.0. Detonation points are found between these stable states when output equals input and slope is

greater than 1.0. Self-limited reverberating discharges of 'tonic' type and relatively fixed duration may be evoked by repetitive supramaximal stimulation sufficient to lower threshold. Progressive increase in threshold during activity terminates the 'seizure.' If intercurrent driving stimuli are continued, the 'tonic' phase of continuous activity is succeeded by a 'clonic' phase of grouped spikes. Observations with anticonvulsant drug treatment and other procedures suggest a limited applicability of this model for studies of seizure phenomena.

Dye dilution curves associated with mitral valvular disease. ROBERT G. TOMPKINS,* HOWARD B. BURCHELL AND EARL H. WOOD. *Mayo Fndn., Univ. of Minnesota, Mayo Clinic, Rochester.*

Theoretically, dye dilution curves with distinctive features might be obtained from persons with abnormal circulations caused by various types of heart disease. Broadbent and Wood, using dye curves continuously recorded by ear and cuvette oximeters, have verified this in persons having congenital defects with intracardiac shunts. This technic was used to compare dye dilution curves from 16 normal subjects with 34 curves from 25 persons with severe mitral disease. Site of dye injection was an antecubital vein. The mean cardiac output (direct Fick) was 2.2 (1.3–3.5) l/min/m.² in the mitral series as compared with a mean value of 2.1 (1.0–32.) obtained simultaneously by the dye method. The standard deviation of the differences was 0.4 l/min/m.² The average values for dye appearance (16 ± 1), mean circulation (30 ± 2), initial dye passage (38 ± 2), and mean systemic recirculation times (34 ± 1) were significantly prolonged in mitral diseases as compared with normal values (14 ± 1, 24 ± 1, 24 ± 2, 21 ± 1 seconds, respectively). However, values in mitral disease did not appear distinctively different when compared with values from normals having similar cardiac outputs. The circulating 'central blood volume' (cardiac output × mean circulation time) and 'lung blood volume' by Newman's method (*Circulation:* 4:735, 1951) were apparently higher in normal subjects (average: C. B. V.: 3.1 (1.7–5.3); L. B. V.: 1.0 (0.4–1.8) than in mitral disease (1.7 (1.0–3.0); 0.8 (0.4–1.6) l., respectively). From this analysis, allowing for level of cardiac output, no distinctive differences were apparent in contour of dye curves recorded from patients with mitral disease and normal subjects.

Effect of administration of ACTH on ammonia content of the brain. CLARA TORDA AND HAROLD G. WOLFF. *Depts. of Medicine (Neurology) and Psychiatry, New York Hosp.-Cornell Med. Center, New York City.*

Administration of ACTH (adrenocorticotrophic hormone of pituitary gland) induces within a few minutes an increase of the electrical activity of the brain consisting of an increase of voltage, occasional spiking, paroxysmal runs of low frequency high voltage waves, and lowering of the threshold for convulsion inducing agents (C. TORDA AND H. G. WOLFF. *Am. J. Physiol.* February, 1952). The mechanism of action of ACTH in inducing the increase of the electrical activity of the brain is not yet known but it seems to be relatively independent of the changes in blood circulation and of the changes in concentration of potassium and sodium. In the following the effect of administration of ACTH on the concentration of ammonia in the brain has been investigated. The ammonia content of the brain was estimated by the method of Conway (microdiffusion analysis). The ammonia content of the brain increased within a few minutes after administration of ACTH. In less than 5 minutes the increase averaged 50%. The ammonia content decreased thereafter and reached values below the resting ammonia content of the brain within half an hour. The amount of the increase of the ammonia content of the brain occurring within a few minutes after administration of ACTH is, at least partially, responsible for the increase of the electrical activity of the brain.

Red cell potassium exchange in sickle cell anemia. D. C. TOSTESON (introduced by R. C. DARLING). *Dept. of Medicine, Columbia Univ., College of Physicians and Surgeons, New York City, and Med. Dept., Brookhaven Natl. Lab., Upton, L. I., N. Y.*

Previous work (D. C. TOSTESON, ETHEL SHEA AND R. C. DARLING. In press) revealed that red blood cells of patients with sickle cell anemia (SCA) lose potassium and gain sodium at roughly equal rates (mEq/hr.) upon de-oxygenation. K loss occurred at a rate of from 10–20 mEq/l.RBC/hr. during the first hour of de-oxygenation. During this period K loss could be reversed by re-oxygenating the red cells. That these disturbances in cation distribution are related primarily to the process of sickling was suggested by the following evidence: *1)* Carbon monoxide, which prevents sickling, also blocked K loss and Na gain by de-oxygenated SCA red cells. *2)* K loss could no longer be reversed by re-oxygenation when the SCA red cells became permanently sickled. Because of the rapidity of the cation shift described above the rate of K exchange in SCA red cells was measured on samples of blood diluted with a K^{42} tagged, glucose enriched, bicarbonate-phosphate buffer incubated at 37°C. Aliquots of red cells, plasma and whole blood taken at appropriate intervals were analyzed for radioactivity, K and H_2O. Results indicated that K penetrates de-oxygen-

ated SCA red cells as rapidly as it penetrates normal red cells. Since the SCA cells show net K loss under these conditions, the absolute outward rate of K movement must be greatly increased.

Myofascial component of intermittent claudication. JANET TRAVELL, SEWARD J. BAKER, BARBARA B. HIRSCH AND SEYMOUR H. RINZLER (introduced by F. GUDERNATSCH). *Dept. of Pharmacology, Cornell Univ. Med. College, New York City.*

Studies on the somatic component of cardiac pain (RINZLER AND TRAVELL. *Am. Heart J.* 35: 248, 1948) have shown that in some patients with angina pectoris and acute myocardial infarction, pain is relieved by blocking secondary trigger areas in the skeletal muscles of the chest. These results in coronary insufficiency suggested that similarly, myofascial trigger areas might mediate the pain of intermittent claudication in occlusive arterial disease of the legs. Preliminary observations on 8 claudicants with advanced arteriosclerosis obliterans, four of whom had diabetes mellitus, indicate that local block of trigger areas in the calf muscles by procaine infiltration and/or ethyl chloride spray may cause marked amelioration of calf claudication pain. Improvement was measured by ergometer (treadlometer), walking or toe-stand tests. Location of trigger areas in the calf muscles and their patterns of referred pain were mapped. Boyd *et al.* (*J. Bone & Joint Surg.* 31B: 325, 1949) likewise found that procaine infiltration of deep trigger points in the calf increased walking distance of claudicants. From these results of therapy one may infer that trigger areas in the calf muscles may themselves interfere with local blood flow by causing reflex vasospasm; even in the presence of occlusive arterial disease, the myofascial component may be the major factor in maintaining a continuing pain cycle. This is in contrast to the generally accepted view that organic changes are mainly responsible for the narrowed lumen of the arterial tree and the ischemic pain of intermittent claudication.

Influence of acetylcholine on synaptic transmission in a parasympathetic ganglion (ciliary). CAROLINE TUM SUDEN, E. ROSS HART* AND AMEDEO S. MARRAZZI. *Neurology Branch, Clinical Research Div., Chemical Corps Med. Labs., Army Chemical Center, Md.*

We (*Federation Proc.* 10: 138, 1951 and *J. Pharmacol. & Exper. Therap.* 103: 364, 1951) have reported successful preparation of the ciliary ganglion in such a way that its circulation remains intact and the influence of blood borne chemicals on the ability of its synapses to transmit impulses can be studied. This is done by using the action potential in the postganglionic fibers as an index of synaptic transmission initiated by uniform repetitive submaximal stimulation of the preganglionic nerve. We have previously reported that these synapses are qualitatively similar to those of sympathetic ganglia, in that they are inhibited by adrenaline and ephedrine. We have recently succeeded in demonstrating the actions of acetylcholine on the ciliary ganglion preparation by injecting appropriate solutions of the drug into the external carotid artery. It was found the actions of acetylcholine on transmission at this site are qualitatively closely similar to those found in other synapses, namely an enhancement of transmission following small doses and an inhibition following larger doses. This fulfills an expected reciprocal relation between adrenergic inhibition and cholinergic excitation at ganglionic synapses. Further tests of the similarity in the nature of synaptic transmission here to that at sympathetic ganglia and to that in the cerebrum are in progress, including the characteristic influence of atropine.

Effect of altered breakfast habits on physiologic responses of men past 60 years of age. W. W. TUTTLE, KATE DAUM* AND BARBARA RANDALL.* *Dept. of Physiology, State Univ. of Iowa, Iowa City.*

The effect of altered breakfast habits on the physiologic responses of a group of 8 men past 60 years of age was studied. The physiologic responses involved were reaction time, neuromuscular tremor, the amount of oxygen required to perform work, and strength. The investigation showed that the omission of breakfast caused a deleterious effect on the physiologic responses of the men. In addition, it was shown that, for the most part, the size and content of the breakfasts studied had no effect on the physiologic responses of the aged group.

Membrane polarization and mechanical responses in an invertebrate smooth muscle. BETTY M. TWAROG (introduced by J. H. WELSH). *Biological Labs., Harvard Univ., Cambridge, Mass.*

Elongate, parallel, unstriated muscle fibers run throughout the length of the anterior byssus retractor muscle of *Mytilus edulis*. A segment of this muscle was depolarized by soaking in isotonic KCl and changes in demarcation potential between the depolarized and the intact portions of the muscle were followed with a millivoltmeter, while mechanical responses of the muscle were being recorded. Acetylcholine and the quaternary ammonium ions, tetramethyl ammonium and 4-ketoamyltrimethyl ammonium, applied to the intact segment, produce depolarization and contraction. Acetylcholine action is potentiated by eserine and blocked by nicotine (after adaptation), by tetraethyl ammonium ion, and, to a lesser degree, by *d*-tubocurarine. Atropine does

not effectively block. Thus the byssus retractor resembles pharmacologically both vertebrate sympathetic ganglia and the heart of *Venus. mercenaria*. Adrenaline and nor-adrenaline produce depolarization and contraction, or no effect, on first application, but after repeated applications, or prolonged application of high concentrations, produce relaxation unaccompanied by any detectable change in membrane polarization. Once this relaxation has occurred, the effect of subsequently applied acetylcholine is altered. The prolonged phase of the acetylcholine contraction is abolished, while the initial contraction and depolarization are potentiated. Ergonovine and tyramine alter responses to acetylcholine in this same manner, without themselves ever producing an initial depolarization or contraction. Evidence was found indicating the physiological significance of the action of acetylcholine on this muscle. Bioassay of muscle homogenates showed an acetylcholine content of the order of one gamma per gram tissue. Manometric determinations indicated significant cholinesterase activity.

Loss of activity of the succino-oxidase system of excised brain tissue in the presence of succinate. DAVID B. TYLER. *School of Medicine, Univ. of Puerto Rico, San Juan.*

In the presence of succinate there occurs a more rapid inactivation of some part of the enzyme system concerned with its oxidation as compared with similar experimental conditions but with the substrate absent. This can be readily demonstrated by adding succinate at various intervals after placing the vessels in the bath and comparing the oxygen uptake with those vessels containing succinate from the start. The addition of small amounts of glucose, lactate or pyruvate prevents the loss in activity of the enzyme system in the presence of succinate. Glutamic acid, HDP, glutathione, cystine and ATP are without effect. DPN accelerates the loss of activity. Preliminary studies indicate that the loss of activity is related to a decrease in—SH groups as determined by the method of Mirsky.

Mechanism of anti-inflammatory action of sodium salicylate. G. UNGAR, E. DAMGAARD* AND F. P. HUMMEL.* *Rheumatic Fever Research Inst., Northwestern Univ. Medical School, Chicago, Ill.*

Recent studies have shown that treatment with Na salicylate causes a depletion of ascorbic acid in the adrenals of intact but not of hypophysectomized rats. On the basis of these observations assumptions have been made that salicylate exerts its anti-inflammatory action by stimulating the pituitary-adrenocortical system (K. C. BLANCHARD *et al. Bull. Johns Hopkins Hosp.* 86: 83, 1950; B. S. HETZEL AND D. C. HINE. *Lancet* 2: 94, 1951). In the course of the present investigation it was confirmed that Na salicylate (100–250 mg/kg.) does cause ascorbic acid depletion in the adrenals of intact rats and that this effect is not observed in hypophysectomized animals. It was, however, also observed that Na salicylate inhibits a standardized experimental inflammation not only in intact animals but also after hypophysectomy, adrenalectomy or splenectomy. The degree of inhibition is approximately the same in all four groups of animals. It seemed likely that in this experimental inflammation salicylate acted directly on some mechanism responsible for the reaction. Furthermore, *in vitro* studies show that Na salicylate inhibits the blood protease, fibrinolysin (plasmin) at concentrations which are at least as low as the effective salicylate level in the blood of the above animals. Since there is some evidence that fibrinolysin may take part in the production of inflammation, the possibility will be discussed that salicylate reduces certain inflammatory processes by inhibiting this enzyme

Forces produced in the thorax by explosive decompression. EDWIN G. VAIL (introduced by J. W. HEIM). *Aero Med. Lab., Wright Air Development Center, Wright-Patterson Air Force Base, Dayton, Ohio.*

The pressures produced by expanding lung gases during explosive decompressions have always presented the possibility of lung pathology. A study was made of the mean pressures produced in the thorax during decompressions. Lateral tracheal pressures were correlated with intrapleural and lateral carotid pressures. An accelerometer rigidly mounted on a mid-thoracic rib gave the time and extent of the maximum force. The time of decompression averaged 150 msec. through a 10-*psi* pressure differential. The subjects were immediately recompressed to avoid hypoxia. The maximum force applied to the thorax occurs in 125 msec. and coincides with maximum intrapleural pressure. The maximum lateral tracheal pressure occurs at 150 msec. or at the decompression time. A shock wave in the circulatory system recorded in the carotid pressure probably resulted from compression on the heart and great vessels. This occurred 20–30 msec. after maximum pressure was reached in the intrapleural space. This shock wave extended 10–20 mm. Hg above the systolic pressure. The intrapleural pressure is greater than the lateral tracheal pressure by 30–40 mm. Hg. Values for thoracic displacement were also obtained from the accelerometer recordings.

Evidence that TSH in dogs produces an early increase of thyroid iodine from plasma forms other than inorganic iodide. JOHN C. VANATTA AND ALLEN F. REID (introduced by ROBERT W.

LACKEY). *Depts. of Physiology and Biophysics, Southwestern Med. School, Univ. of Texas, Dallas.*

The effects of injecting 8 Junkmann-Schoeller units of thyroid stimulating hormone (TSH) on the I^{131} content of the thyroid have been observed. In 7 experiments on 3 dogs, which had been given I^{131} 72 hours or more previous to TSH (Armour) injection, there was found an increase in content of I^{131} within 6 hours after the TSH. Since the increase occurred more quickly than reported increases in iodine uptakes following TSH, this was studied further. The phenomenon could not be consistently produced in dogs given inorganic I^{131} 3 hours or less prior to the administration of TSH. However, when a donor dog was given 500 microcuries I^{131}/kg., heparinized plasma obtained from this animal 3 days later was given intravenously to another animal and the recipient given 8 units TSH, then there was an early increased uptake of I^{131} similar to that found in the first three animals. In 5 animals uptakes were measured after plasma, after plasma and TSH, and after inorganic iodide and TSH. All dogs showed increased I^{131} content in the thyroid within 6 hours after TSH following plasma injections, whereas only one of the 5 showed such an increase after TSH following inorganic I^{131} injections. These data support the hypothesis that iodine in some form other than inorganic iodide can reenter the thyroid gland.

Iodide metabolism in rats on low iodide intake. LESTER VAN MIDDLESWORTH. *Div. of Physiology, Univ. of Tennessee, Memphis.*

Rats have been raised on a diet containing 20 to 30×10^{-6} gm. iodide/kg. (analyzed repeatedly for iodide content). Thirty days after weaning rats to this diet a fixed small amount of an I^{131} solution was added each day for 30 additional days. The animals were fed 10 gm. of diet/rat/day. The radioactivity of the PBI fraction of plasma rose continually. After one week 90% of the daily I^{131} intake was excreted in the urine and 7% in the feces. The plasma PBI fraction ultimately contained a maximum of 5% of the daily ingested I^{131}/cc. and the plasma iodide was 0.16% to 0.50% of the daily I^{131}/cc. plasma, this indicated a plasma ionic iodide in the order of 0.1 to 0.3×10^{-6} gm/100 cc. The weight of the thyroid glands remained normal even after 18 months on the diet, and the chemical PBI levels were normal. At the end of this period the thyroid glands contained 6–10% of the daily I^{131} intake. These rats all had thyroids of normal weight (9 mg/100 gm. body wt.). When 0.5 mg. thiouracil was added to the daily diet the thyroid size increased to 18 mg/100 gm. rat. Then when 100 gamma iodide plus 0.5 mg. thiouracil was added to the daily diet the thyroids were normal in size (7.5 mg/100 gm. rat). Therefore, it is difficult to produce symptoms of iodine deficiency by very low iodide intake unless a goitrogen is present in the diet. Even so, a dietary goitrogen may be titrated with iodide intake to become ineffective.

Effect of thyroxine on the thyroid. LESTER VAN MIDDLESWORTH. *Div. of Physiology, Univ. of Tennessee, Memphis.*

Rats were placed on a diet containing 20 to 30×10^{-6} gm. iodide/kg. The diet was tagged with I^{131} (initially 400,000 c/m/10 gm. diet) and the animals approached equilibrium on the radioactive diet. The rats were divided into 3 groups and placed in metabolism cages. Into one group 0.03 mg. thyroxine was injected each day, into another group an equivalent amount of KI (2 gamma) was injected each day, the third group served as controls. All 3 groups were fed the radioactive diet for 11 days. The rats injected with thyroxine showed a rapid decline in PBI^{131} (considered to represent endogenous PBI) to 10% of that found in controls. The 2 gamma/day of KI slightly reduced the PBI^{131} of the second group. The thyroid glands of the thyroxine injected rats contained 3 to 5 times as much iodide as the controls. Evidently exogenous thyroxine quickly and quantitatively stopped the PBI output of the thyroid gland.

Menstrual cycle after androgen treatment. GERTRUDE VAN WAGENEN. *Dept. of Obstetrics and Gynecology, Yale Univ. School of Medicine, New Haven, Conn.*

Testosterone propionate will prevent the bleeding which terminates the normal menstrual cycle in the rhesus monkey (HARTMAN, 1937) and will suppress the physiological activity of the ovary (ZUCKERMAN, 1937). Paradoxically, it will also bring the very young monkey into a state of sexual maturity (menarche) at one year of age (VAN WAGENEN, 1950), although the menstrual cycles in this species usually begin at the end of the second year. No permanent injury to the ovary of this animal has been found at the hormone levels used. Of 4 young females, 3 were injected with 7.5 mg/kg/week from the fifth month to about a year of age. Two were found to have menstruated, the first at 11 months, 26 days and the second at 1 year and 20 days. These were not spurious bleedings but the first of cyclic bleedings. A fourth animal received 10 mg/kg/week from the age of 9 months to 14 months, menstrual cycles beginning after an interval of 10 days. Three monkeys were treated for longer periods extending into the adult years. Presumably, these animals were hastened to maturity initially and continued treatment suppressed the ovaries. Testosterone administration (2.5 mg/kg/week, but increased in the second and third monkey to

7.5 mg/kg/week) began when they were 2 months old and ended at 2½ years, 3 years and 3½ years. After injections were stopped, intervals of 81, 63 and 62 days occurred before menstrual cycles were established with accompanying sexual skin development and vaginal desquamation. In both groups, those made sexually precocious and those with delayed menarche, individuals which were mated became pregnant, indicating that full ovarian function was not impaired.

Comparison of DFP- and methylene blue-induced anemias. A. B. VIALPANDO,* H. C. STRUCK AND L. H. HOEBELHEINRICH.* *Dept. of Physiology and Pharmacology, Creighton Univ. School of Medicine, Omaha, Neb.*

Two dogs have been given daily oral doses of 60 mg. methylene blue per kg. Another dog has been given daily intravenous doses of 0.5 mg. DFP/kg. In the dogs receiving methylene blue, a decrease of 5 million in erythrocyte count was noted within one week. Reticulocyte values rose to 12 to 18%. Erythrocyte fragility was markedly increased. Cholinesterase activity of the erythrocytes showed a slight decrease at first, rose to high values as the reticulocyte response became evident, and returned to normal levels along with the reticulocyte count upon withdrawal of the dye. Bone marrow differential studies showed a decrease in the leuko-erythroid ratio from 4.09:1 to 0.58:1. In the dog receiving DFP, a gradual decrease of two million in erythrocyte count occurred within 10 days. No further decrease was obtained despite continued administration of larger doses of drug. Erythrocyte cholinesterase fell to 30% of normal values, and a further decrease came about only after increasing DFP dosage. Reticulocyte values did not rise above 2% at any time. Fragility of the cells did not change. Bone marrow studies showed a decrease in leuko-erythroid ratio from 4.85:1 to 1.48:1. These experiments are being expanded and repeated, but these results indicate that anemia produced by DFP is not of the severe hemolytic type produced by methylene blue, and that inhibition of erythrocyte cholinesterase by methylene blue, which has been shown to occur *in vitro* is not the principal cause of the anemia produced by the dye.

Increase of reflex time during growth. I. H. WAGMAN. *Dept. of Physiology, Jefferson Med. College, Philadelphia, Pa.*

On the basis of results of studies of conduction velocities of ulnar nerve motor fibers in humans of various ages and sizes, Wagman and Lesse (*J. Neurophysiol.*, in press) concluded that the time consumed in the efferent pathway of a reflex arc increases during growth from the age of 5 years onward. Recently Rushton (*J. Physiol.* 115: 101, 1951) in postulating "A theory of the effects of fiber size in medullated nerve" stated as one of his conclusions "that the duration of peripheral conduction time in the reflex arc will remain constant during growth." Our present studies made on a reflex arc extend our own conclusions. Measurements were made on human subjects of various ages and sizes, of the reflex response (action potential) recorded from the calf muscles following percutaneous electrical stimulation of the tibial nerve in the popliteal fossa. In every experiment all electrodes were placed in comparable positions. It was found that the latent period (time between stimulation to nerve and beginning of reflex action potential) increased during growth. In two typical experiments, one on a 5-year-old boy, 42 inches tall, the other on a 25-year-old man, 58 inches tall, the respective latent periods were 19 and 32 msec. These results are explained again by the facts that maximum fiber diameter is achieved at an early stage of growth, and that during most of growth while the nerve fiber is increasing in length, its diameter and conduction velocity remain constant. (Aided by a grant from the National Institutes of Health.)

Factors contributing to renal enlargement following ureteroduodenostomy and contralateral nephrectomy.* KHALIL G. WAKIM, MELVIN A. BLOCK* AND FRANK C. MANN. *Div. of Exptl. Medicine, Mayo Fndn. Univ. of Minnesota, Rochester.*

In dogs and albino rats one kidney was removed and carefully weighed and the ureter of the remaining kidney was transplanted into the duodenum. This kidney had to re-excrete the reabsorbed material which it had already eliminated into the intestine. In another series of animals one kidney was removed but the contralateral kidney and ureter were left intact. At definite intervals the remaining kidney in each series was removed and its weight, volume and histologic condition were compared with those of the control kidney that was removed at the start. The intact kidney in rats after unilateral nephrectomy enlarged from 0 to 15% over the control kidney that had been removed 1 to 6 days previously, whereas in dogs it enlarged from 0 to 55% in 10 days. There were a few mitotic figures in the renal parenchymal cells. However, in the series in which the ureter of the remaining kidney was transplanted into the duodenum enlargement of the kidney was rapid and progressive. The kidney enlarged 100% or more in volume and weight as compared to the control contralateral kidney that was removed at the start of the study. The dogs and rats in this series had a great rise in blood urea and died in uremia, the dogs within 12 days and the rats within 6 days after the nephrectomy and contra-

lateral ureteroduodenostomy. Microscopic examination demonstrated the presence of a much greater number of mitotic figures in the nephrons of the kidney whose ureter was transplanted into the intestine than in the other series. The augmentation in size and weight of the kidney is due to an increase in the intratubular, interstitial and intracellular fluid, in the number of parenchymal cells, as well as in the capacity of the renal vasculature. The enlargement could be partially or completely prevented by marked constriction of the renal artery.

Relation of increased body temperature to ventricular fibrillation induced by ether inhalation in the rat. SHEPPARD M. WALKER. *Dept. of Physiology, Univ. of Louisville School of Medicine, Louisville, Ky.*

Mature female rats of the Anheuser-Busch strain were placed in the supine position and restrained. In the unheated control animals the rectal temperature varied from 37° to 39°C. The experimental animals were placed under a heating lamp and warmed slowly until the rectal temperature reached 41° to 42°C. This temperature increase was maintained throughout the study with ether. Ether was given in all cases until respiration ceased. In those experiments not showing ventricular fibrillation breathing was promptly restored by artificial respiration. After a 15-minute recovery period ether administration was repeated. If ventricular fibrillation did not occur within 4 periods of ether inhalation, in a given animal, experiments were discontinued. Electrocardiograms were obtained with a Sanborn visocardiette recorder. Ventricular fibrillation was verified in the exposed heart. Ventricular fibrillation developed in all of 7 animals with the body temperature increased to about 42°C. The onset of fibrillation occurred before respiration ceased in 3 of the 7 animals. The average duration of exposure to ether in the experiments showing fibrillation was 40 seconds. Ventricular fibrillation developed in one of 8 unheated control animals. Under the conditions of these experiments an increase of body temperature increases the incidence of ventricular fibrillation by ether inhalation.

Effect of amino acids on cholic acid synthesis in bile fistula dogs. G. WALTER* AND J. H. ANNEGERS. *Dept. of Physiology, Northwestern Univ. Med. School, Chicago, Ill.*

Previous work in this laboratory has failed to demonstrate cholepoietic activity from the intraduodenal administration of enzymatic hydrolysates of casein. Other investigators, however, have reported that the parenteral administration of a mixture containing the ten essential amino acids plus glycine stimulates cholate synthesis. Accordingly, this latter mixture was tested under our experimental conditions. Total daily cholate outputs were determined (IRWIN, JOHNSON, AND KOPALA. *J. Biol. Chem.* 153: 439, 1944) during 3-day periods in external bile fistula dogs receiving *a*) saline, *b*) saline plus 50 gm. of enzymatic casein hydrolysate, and *c*) saline plus 50 gm. of the amino acid mixture per day. On these respective regimes the mean daily cholate outputs averaged *a*) 622, *b*) 677, and *c*) 1095 mg. in 7 dogs. Statistical analysis of these data revealed no significant effect from the enzymatic hydrolysate, whereas the other amino acid mixture yielded significantly greater outputs than the saline control or the enzymatic hydrolysate. These results suggest that an amino acid(s) is involved in cholepoiesis, and that this acid(s) is destroyed or inactivated by an enzymatic digestion.

Plasma and dextran infusions following hemorrhage in dogs. KARLMAN WASSERMAN AND H. S. MAYERSON. *Dept. of Physiology, Tulane Univ. School of Medicine, New Orleans, La.*

Dogs, anesthetized with pentothal, are bled so that their blood pressures remain at or about 40 mm. Hg for 30 minutes to 1 hour. The blood removed is replaced by equivalent amounts of canine plasma or 6% clinical dextran. Severe hemorrhage *per se* results in a loss of plasma protein greater than can be accounted for in the blood removed. This loss amounts to 20 to 25% of the prehemorrhage total circulating protein and is usually associated with an initial fall and subsequent rise in thoracic lymph flow and the appearance of erythrocytes in the lymph. Infusion of isotonic plasma results in a retention of only 50 to 60% of the infused volume 30 minutes after the end of the infusion, with a proportionate decrease in total circulating plasma protein. By contrast, 30 minutes after dextran infusions, all of the infused volume is retained and no significant gain or loss of protein occurs which can not be accounted for in the thoracic duct lymph. The ratio of plasma dextran to lymph dextran is greater than the plasma protein to lymph protein concentration ratio. At 30 minutes, 2 hours and 4 hours after infusion, more of the dextran remains in the circulation than does infused plasma protein. Lymph flow usually increases following the two types of infusions. The initial dilution of plasma protein by dextran infusion results in a level of protein which is below that of thoracic lymph. (This work was done under contract DA-49-007-MD-38 between Tulane Univ. and the Dept. of the Army.)

Rotor temperature measurement and control in in ultracentrifuge. DAVID F. WAUGH AND DAVID A. YPHANTIS.* *Biology Dept., Massachusetts Inst. of Technology, Cambridge.*

Moving rotor temperatures may be measured

with a precision of ±0.03°C. and an accuracy of 0.1°C. in a vacuum centrifuge. The method does not use physical contact with the rotor but utilizes the infra-red radiation from the rotor. A reference temperature is maintained in a mass of metal (reference block) placed near and shielding the base of the rotor. A copper disc, shielded also by the reference block, assumes a temperature between rotor and reference block temperatures through radiation exchange. The various temperatures and temperature differentials are determined by copper-constantan thermocouples. Heat conduction through thermojunction leads and copper disc supports, and radiation exchange between copper disc and reference block have been minimized; the copper disc maintaining a temperature 0.72 of that between reference block and rotor. On acceleration the rotor cools, the opposite effect occurring during deceleration. Such changes are shown to be due to the adiabatic changes concomitant with the production or release of stress in the rotor. At 60,000 rpm a change of −0.9°C. is observed on acceleration. Such changes have not generally been taken into account. By installing a chamber heating coil in addition to refrigeration, the moving rotor temperature may be kept to within ±0.05°C. of a chosen temperature for indefinite periods.

Persistence of active vasomotion after denervation (motion picture). R. L. Webb* and P. A. Nicoll. *Depts. of Anatomy and Physiology, Indiana Univ. School of Medicine, Bloomington.*

The well-known vascular responses following nerve stimulation, especially of sympathetic fibers, along with the often demonstrated vascular reactions elicited by, or associated with, reflex mechanisms have given rise to the generally accepted idea that the motor reactions of the peripheral vessels are controlled by nervous means. Nevertheless considerable evidence exists which indicates that motor responses of peripheral vessels do not require a neural mechanism. The film shown demonstrates that the vascular behavior of both blood and lymphatic vessels known as active vasomotion is to a great degree independent of neural control. These studies were made on the wing membrane of the common bat, myotis leucifugus, where subcutaneous vascular behavior may be easily studied. The entire field may be denervated by a simple operation and the animals maintained for study without difficulty. The film first demonstrates the marked decrease of vascular response to direct nerve excitation as one moves peripherally along the arterial tree. There appears to be a sudden lessening of the control between the small arteries and terminal arterioles. The remaining portion of the film shows the typical behavior of the arteriolar vessels, lymphatics, and veins on various days following complete denervation. Little change in their typical reactions are observed until towards the 21st post operative day. From then on the terminal arteriole shows progressively a tendency to maintain a contracted state and individual muscle cells along the vessels become erratic in their behavior.

Effect of arteriovenous fistula on circulation. René Wégria, Charles W. Frank,* Hseuh H. Wang* and Robert Miller.* *Dept. of Medicine, Columbia Univ. College of Physicians and Surgeons, and Presbyterian Hosp., New York City.*

Dogs were anesthetized with chloralose and their chests were opened through a midsternal incision. Under artificial respiration, 2 rotameters were inserted into the arterial side of the systemic circulation. The first one, inserted according to a technique previously described, recorded the output of the left ventricle with the exception of the amount of blood flowing through the arteriovenous fistula which was recorded by the second rotameter. The magnitude of the fistula established between the root of the aorta and the proximal end of one of the femoral veins could be adjusted by a tunnel-clamp. Mean arterial blood pressure was recorded simultaneously with the amount of blood flowing through the fistula and the 'body flow,' i.e. the output of the left ventricle minus the fistula flow. The fistulas were established progressively, maintained for 5 minutes, then suddenly occluded. The results observed can be summarized as follows: With 'small' fistulas, i.e. fistulas accomodating a blood flow of up to 20% of the control cardiac output, generally neither the 'body flow' nor the mean arterial blood pressure was modified and the cardiac output was increased by an amount equal to the fistula flow. With 'large' fistulas, i.e. fistulas accommodating blood flow up to around 60% of the original cardiac output, the increase in cardiac output which occurred was greater than with 'small' fistulas, but the 'body flow' fell below its control level; as shown by an increase in the total peripheral resistance, vasoconstriction occurred and the mean arterial blood pressure fell only slightly. With 'very large' fistulas, the increase in cardiac output was still greater; the 'body flow' decreased markedly and, despite a marked vasoconstriction, an important decrease in mean arterial blood pressure occurred.

Some variables in serum prothrombin activity. Murray Weiner and Niel Wald (introduced by William Goldring). *Third New York Univ. Med. and Research Services, Goldwater Memorial Hosp., Welfare Island, N. Y.*

Recent studies of prothrombin consumption have demonstrated that deficiencies in the anti-

hemophilic globulin (thromboplastinogen) or platelet-tissue thromboplastic enzyme may influence the conversion of prothrombin to thrombin. The estimation of prothrombin consumption is complicated by considerable variation in serum prothrombin activity under different conditions of incubation and separation of serum from the clot. An investigation of these variations has demonstrated the following: *a*) Serum separated as soon as normal blood clots yields an apparent serum prothrombin time equal to or shorter than that of the original plasma, and loses that activity slowly on subsequent incubation. *b*) If the separation is delayed for several minutes, there is considerable loss of prothrombin activity on subsequent incubation of the serum. *c*) If a period of about 1 hour or more is allowed to elapse before the serum is separated, the prothrombin activity of the subsequently incubated serum remains relatively constant. Reproducible, consistent serum prothrombin estimations are obtained only after this relatively stable state is reached. These 3 phases of serum prothrombin activity are suggestive of the presence of a substance which develops as clotting progresses, and interferes with prothrombin activity of serum if separated with the serum. If the serum remains with the clot, this substance is apparently adsorbed by the clot. This sequence of event suggests that the substance involved may be thrombin.

Utilization of radioactive iodide by cell-free preparations of beef thyroid tissue. BENJAMIN WEISS (introduced by DAVID J. SANDWEISS). *Research Div., Harper Hosp., Detroit, Mich.*

The utilization of radioactive iodide (I^{131}) in the formation of diiodotyrosine and thyroxine by surviving thyroid slices was first demonstrated by Morton and Chaikoff (*J. Biol. Chem.*, 147: 1, 1943). It was shown in their studies that the intact cell was required for maximum conversion, since homogenization of the tissue prior to incubation resulted in failure to incorporate added I^{131}. In the present investigation, it was found that homogenates were able to utilize I^{131} in the formation of diiodotyrosine and thyroxine when supplemented with copper and tyrosine. The addition to the complete system either individually or in combination of ATP, Ca, Co, cysteine, cytochrome c, DPN, F, Mg, Mn, nicotinamide, pantothenic acid, pyridoxine, thiamine, or dried brewers yeast, had no augmenting effect on uptake. Although incubation of the slice in the presence of copper depressed the uptake of I^{131}, copper was required by the homogenate for maximum activity. In a similar manner, anaerobiosis, which almost abolished the conversion of I^{131} by the slice, exerted no comparable effect on the homogenate. Dialysis of the homogenate resulted in a progressive loss in activity. Differential centrifugation disclosed that the enzymes involved in the synthesis of thyroxine are distributed in the nuclei and mitochondria, the latter exhibiting the greatest activity. With mitochondria as the enzyme source, the presence of cytochrome c or DPN had an inhibitory effect on the utilization of I^{131}. The same system that was capable of converting I^{131} into the organically bound form was unable to esterify inorganic P^{32}, thus indicating that phosphate bond energy is not directly coupled to the biosynthesis of thyroxine.

In vivo phenomena suggesting a heparin-lipoprotein reaction. C. B. WELD AND J. J. SPITZER.* *Dept. of Physiology, Dalhousie Univ., Halifax, Canada.*

Very small doses of heparin (20 U/kg.) given intravenously to dogs cause a number of phenomena that can best be explained on the basis of a reaction with the lipo-protein mechanism. We have found that after giving these small doses to fasting or to lipemic dogs: *1*) The number of chylomicrons is strikingly reduced; clumping occurs. At the same time, in lipemic animals the free and combined lipids are usually, but not always, reduced. *2*) Hemolysis is produced in lipemic but not in fasting animals, due to a decreased antihemolytic power of the plasma. *3*) The coagulation time is slightly prolonged. The lipemic state itself usually shortens but occasionally prolongs the clotting time; in the latter cases the heparin hastens clotting. *4*) The thromboplastin concentration is reduced (prothrombin-consumption test of Quick). *5*) Prothrombin time is unchanged. *6*) The thrombin inactivation rate (Gerendàs), usually increased, is sometimes sharply reduced. It should be noted that the above reactions are obtained by intravenous heparin, and that in 2 of them the state of lipemia qualitatively affects the results. The usual, larger doses of heparin regularly cause a prolonged clotting time, a prolonged prothrombin time, and an increased anti-thrombin activity. Chargaff has shown that heparin can split the lipoprotein molecule and combine with the protein moiety. It is suggested that the effect of the small doses is to initiate changes in the lipo-protein complex that may require larger doses for completion.

Bromsulphalein and cardiac output in hepatectomized dog. ATTIE YVONNE WERNER* AND STEVEN M. HORVATH. *Dept. of Physiology, State Univ. of Iowa, Iowa City.*

Sixteen anesthetized dogs were hepatectomized according to the method of Firor and Stinson, with several modifications. Cardiac output by the Fick principle was estimated prior to and following hepatectomy in 8 dogs. The rate of removal of bromsulphalein was measured in all dogs. In 5

animals bromsulphalein was injected and infused, in the remaining animals bromsulphalein was injected only. The plasma concentrations of bromsulphalein were in the range normally used to measure hepatic blood flow. T1824 (Evans Blue) was injected into 10 animals to determine plasma volume and to follow its rate of disappearance. Bromsulphalein was read at 580 mu and T1824 at 620 mu in a DU Beckman spectrophotometer. The disappearance of T1824 after hepatectomy was within normal ranges for intact animals. Bromsulphalein disappeared from the blood of all hepatectomized dogs. The rate of disappearance varied from animal to animal, ranging from 20 to 50, averaging 35% in the first hour after hepatectomy and continuing thereafter as long as the animal survived. The loss of bromsulphalein in the urine was less than 0.20% in every case. Following hepatectomy cardiac output was reduced, oxygen consumption decreased and the carbon dioxide content of the blood decreased. However, the normal rate of disappearance of T1824 suggested that the extrahepatic loss of bromsulphalein was not affected by the markedly altered state of the animal. Determinations of hepatic blood flow by the bromsulphalein method must be a qualitative measurement due to the removal of the dye by the liver plus extrahepatic loss.

Effect of mercurial diuretics during Pitressin antidiuresis in man. R. E. WESTON, J. GROSSMAN,* E. R. BORUN,* AND L. LEITER.* *Med. Div., Montefiore Hosp., New York City.*

To analyze further the renal site and mechanism of mercurial diuretic action, the effect of mercurials during Pitressin antidiuresis was studied. In fasting human subjects profuse diuresis was produced by repeated oral administration of 1000–1200 ml. of water/hour. After starting isotonic saline or lactate-Ringers infusions, to prevent electrolyte depletion, urine was collected until urine flow had reached a constant maximal rate. Then, 1–3 mU Pitressin/kg. body weight were injected intravenously, and equivalent quantities given/hour in the infusion. When urine volumes dropped to minimal levels, generally from 0.6–1 ml/minute, 2 ml. of mercurial (Thiomerin) were given intravenously until maximal diuresis occurred. Blood for determination of serum electrolyte concentration was drawn periodically. During water diuresis, urinary electrolyte concentrations were low and excretion rates relatively constant. After Pitressin, urinary electrolytes became hypertonic but sodium and chloride excretion increased somewhat or remained constant. Following the mercurial, there was profuse water diuresis with significant fall in urinary electrolyte concentration despite the marked increase in sodium and chloride excretion. As sodium and chloride diuresis subsided, urinary volume declined but sodium concentration increased again. After Pitressin, potassium excretion remained unchanged or decreased, but following mercurial administration, usually decreased. The significance of the dilution of the hypertonic post-Pitressin urine with less concentrated, perhaps isotonic, solution of sodium and associated anions during mercurial diuresis will be discussed.

Investigation of conditions favoring cardiac arrest. C. W. WHITE, JR. AND MARTIN RYAN (introduced by GEORGE L. MAISON). *Dept. of Pharmacology, Boston Univ. School of Medicine, Boston, Mass.*

Because of the number of clinical reports of cardiac arrest during anesthesia, it is of interest to investigate the circumstances which favor this occurrence. Preliminary experiments have been performed to determine the influence of ether, hypoxia and procaine in stopping the heart. Studies on 18 dogs have indicated great variability in susceptibility. In keeping with unpublished observations of Maison *et al.*, it was uniformly observed that the combined influence of these three factors caused respiratory arrest. In addition, the peripheral circulation was rendered ineffective, but the development of cardiac arrest was unpredictable. Under ether anesthesia 9 of 18 dogs responded with cardiac arrest (as evidenced by a flat EKG record of 2-min. duration) within 3 minutes following the respiratory arrest produced by procaine (10–15 mg/kg.) or hypoxia (5% O_2 breathing mixture) or both. Another 4 animals developed cardiac arrest at 5, 7, 20 and 22 (*sic*) minutes following respiratory arrest. Of the 5 remaining animals none developed a completely flat EKG, but no cardiac contractions were observed on direct visualization of the heart; furthermore, blood pressure tracings at that time revealed no arterial pulsation in these dogs. Attempts were made to resuscitate 12 animals by means of cardiac massage and/or epinephrine. Effective circulation was restored in 5, and in 7 cases ventricular fibrillation resulted.

Relation of size of shock dose of antigen to blood pressure fall and heparin release in canine anaphylaxis. RICHARD P. WHITE AND PARKE H. WOODARD (introduced by O. O. STOLAND). *Dept. of Physiology, Univ. of Kansas, Lawrence.*

It is well known that both fall in blood pressure and heparin release are phenomena appearing in canine anaphylaxis. It has been shown by others that heparin does not invariably appear in the blood in all cases even when blood pressure falls markedly. In previous work we have reported that heparin often appears in the thoracic duct lymph when not appearing in the blood. In connection with this work we have been able to show

that whether or not heparin appears in either blood or lymph is in a measure related to the size of shock dose of antigen used. Two separate antigens have been used—horse serum and hemocyanin. When relatively large doses of horse serum (0.75–1.0 cc./kg. of body weight) were used, in 9 of 10 dogs blood pressure fall was accompanied by significant quantities of heparin in lymph. In 3 of these animals minute quantities only were present in the blood. When the dose of horse serum was kept below 0.1 cc/kg. in 12 dogs, blood pressure drop was accompanied by significant heparin release in the lymph in 4 animals, and in the blood in only 1. Increasing the dose of antigen to somewhat above 0.1 cc/kg. body weight in general increases the likelihood that heparin will appear in the lymph, but the relation is not directly proportional. The hemocyanin results in general parallel those obtained with horse serum, but indicate the former to be more effective than horse serum as an antigen in canine anaphylaxis.

Physical properties of lungs in experimental pulmonary edema. JAMES L. WHITTENBERGER, JOHN E. AFFELDT* AND JERE MEAD.* *Dept. of Physiology, Harvard School of Public Health, Boston, Mass.*

The time course of changes in pulmonary elastance and resistance has been followed in intact tracheotomized dogs during temporary elevation of pulmonary vascular pressures and during the production of pulmonary edema by saline infusion and other methods. In a first series of dogs striking changes in elastance were evident in a series of static pressure-volume diagrams. In a second series the changes were observed instantaneously by tracing the oscilloscope plot of tidal volume related to change of intrapleural pressure. Tidal volume was obtained by electrical integration of the signal from a low resistance flowmeter. Changes of intrapleural pressure were obtained from an esophageal balloon. From simultaneous recordings of air flow, volume change, and pressure change, pulmonary resistance, as well as elastance, was calculated. Under certain conditions the resistance could be plotted continuously on the oscilloscope. With rapid saline infusions there was a continuous diminution of pulmonary distensibility; this effect was reversed when the infusion was stopped. Brief overinflation temporarily increased distensibility and forced deflation reduced distensibility. Analogous alterations of distensibility occurred when similar changes in lung volume were brought about by use of artificial respiration devices producing intermittent positive airway pressure or alternating positive and negative pressure. Sudden mechanical obstruction of pulmonary venous outflow in otherwise normal dogs produced a decrease in distensibility for the duration of obstruction.

Relation of pH to the shortening of muscle protoplasm by cations. FLOYD J. WIERCINSKI. *Dept. of Physiology, Hahnemann Med. College, Philadelphia, Pa.*

Preliminary measurements with the microinjection of Na sulfonphthalein pH indicators show that when a muscle fiber with cut ends is immersed in 0.2M $NaHCO_3$, the intracellular pH is 8.4, in 0.12M NaCl, pH 6.0, in mineral oil, pH 5.8–6.4 (6.0), and in 0.186M NH_4Cl, pH 5.6. Into such fibers various salt solutions were injected and the contraction studied. Contraction is expressed as a + value, relaxation as the − value. When the muscle fiber is immersed in 0.186M NH_4Cl, and 0.1M NaCl is microinjected the mean value for contraction is −2.4%; at 0.2M −9.50%; at 0.3M +1.8%; at 0.4M −14.0%. For 0.1M KCl −5.2%. For 0.08M MgCl −5.2%. For 0.002M $CaCl_2$ +51.2%. When the muscle fiber is immersed in 0.12M NaCl, and 0.1M NaCl is microinjected the mean value for contraction is +19.0%; at 0.2M +4.0%; at 0.3M +9.0%; at 0.4M +14.0%. For 0.1M KCl −1.45%. For 0.08M $MgCl_2$ −2.02%. For 0.002M $CaCl_2$ +46.5%. When the muscle fiber is immersed in 0.2M $NaHCO_3$, and 0.1M NaCl is microinjected the mean value for contraction is −31.5%; at 0.2M −13.3%; at 0.3M −52.6%; at 0.4M −31.4%. For 0.1M KCl −6.1%. For 0.08M $MgCl_2$ −7.2%. For 0.002M $CaCl_2$ +35.4%. These data suggest that 0.002M $CaCl_2$ is effective from pH 5.6 to 8.4 to elicit contraction in the muscle fiber whereas $MgCl_2$, NaCl, and KCl, at intracellular pH's other than 6.0–6.4 relax the muscle fiber.

Effect of acetylcholine on a stretch receptor of crayfish. C. A. G. WIERSMA, E. FLOREY,* AND E. FURSHPAN.* *Kerckhoff Labs. of Biology, California Inst. of Technology, Pasadena.*

Alexandrowicz (*Quart. J. Micr. Sc.* 92: 163, 1951) has recently described muscle-spindle-like receptors in two decapod crustacea. In the present investigation, these were also found to be present in the abdomen of the crayfish, *Cambarus clarkii*, though with minor differences in their anatomical arrangement. The 2 receptors, which are located on each side of each abdominal segment, have been shown to respond to stretch in a rather typical manner, though differing from each other in threshold and in rate of adaptation, the larger having the higher threshold and the quicker adaptation rate. One of the receptors may be removed so that an organ giving to sensory potentials of a single rhythm may be studied. Dilute solutions of acetylcholine have been shown to increase the activity of these sense organs, a noticeable effect being obtained in stretched preparations with

dilutions of 1×10^{-8}. Higher concentrations may initiate activity in relaxed preparations. The effect of acetylcholine is not potentiated by eserine ($1 \times 10^{-6} - 2 \times 10^{-4}$).

Electrolytes in muscle of rat forelimb after intense local x-irradiation. W. S. WILDE AND C. W. SHEPPARD. *Dept. of Physiology, Tulane Univ., New Orleans, La., and Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.*

Wistar adult male rats under Nembutal were secured under a 13-mm lead plate shield containing a window for exposure of the muscles of one forelimb (elbow to wrist) to x-rays (single doses, 250 kvp, 10–73 kr; rate 720 r/min. 30 mm. from shutter and filtered through 3 mm. of aluminum; scatter to other parts of animal 0.11% of dosage). Companion muscle from the other shielded forelimb served as control against indirect influences which might arise from whole-body effects of scattered radiation. For many hours after exposure, the animal continued to use the limb freely. The rayed muscle, sampled 22 hours after exposure began, was swollen but bore no other visible evidence of damage, and remained functional. All analyses were expressed per 250 gm. dry weight of muscle completely cleaned of fat, membranous and tendinous connective tissue. Changes would otherwise be masked by swelling. Total water of the exposed muscle increased with dosage to exceed controls by 25%. Potassium content remained unchanged. Simultaneous inulin and Na^{24} spaces in a separate group of rats, nephrectomized at 22 hours to prevent urinary loss of inulin, proved that the water gain was entirely interstitial. Gain in total water and gain in Na^{24} and inulin space were all three equal. Shielded control muscles from all rats collectively showed no changes correlated with total body irradiation such as might occur from scatter. Thus water, potassium, and sodium were unchanged in exposed muscle fibers but edema occurred because of *local* vascular disturbances.

Emotional blood pressure responses of trained normal dogs. C. M. WILHELMJ, E. B. WALDMANN,* T. F. MCGUIRE* AND JOHN MCDONOUGH.* *Dept. of Physiology and Pharmacology, Creighton Univ. School of Medicine, Omaha, Neb.*

Daily blood pressure determinations have been made on 14 trained dogs over long periods of time. The various manipulations have been highly standardized, with the result that the dogs have become conditioned to the procedures. This report concerns the effect of certain accidental or planned innovations in the procedure such as: *1*) a change in routine; *2*) strangers in the laboratory; *3*) replacing the operator who trained and usually made the readings by another; *4*) previous noise and great confusion in the kennels; *5*) presence of a female in estrus. One group of animals seemed quite well balanced and phlegmatic and gave virtually no response to these minor changes, whereas another group responded by marked elevations in blood pressure which were grossly excessive in view of the mildness of the emotional stress. Actual gross muscular movements or struggling were not observed and cannot be considered a cause. Aside from the natural differences in the responsiveness of the animals, the degree of training seemed to be an important factor, in that a high degree of training seemed to be conducive to greater emotional instability. These findings suggest that many common laboratory procedures may incite considerable emotional tension in trained dogs which is not grossly evident. It is provocative to ask whether the restraints and conditioning characteristic of modern civilization result in a similar degree of vasomotor sensitivity to minor environmental and emotional stimuli in human subjects.

Myocardial lesions in mice fed a yeast-protein diet. W. LANE WILLIAMS AND RICHARD B. ARONSOHN.* *Dept. of Anatomy, Univ. of Minnesota, Minneapolis.*

Cardiac lesions were observed in all of 43 hybrid mice (Strong A x Bagg albino) fed a (British) yeast-protein diet (HIMSWORTH AND GLYNN) for 64–283 days. The mice were restricted to this diet when 6–10 weeks of age. Ceroid pigment was generally present in all of the hearts. Eighteen mice showed extensive necrosis and fibrosis of the myocardium, and 20 showed punctate necrosis without significant fibrosis. Myocardial damage was present in 8 mice that died after receiving the diet for 60–80 days. No cardiac lesions were observed in a very small group of mice (4 animals) fed the same diet for 60–80 days but receiving 2 mg. alpha tocopherol weekly by intraperitoneal injection. These results indicate that myocardial lesions are more frequent than extensive hepatic necrosis in mice restricted to a diet in which yeast is the source of protein. Preliminary studies indicate that deposition of ceroid pigment and necrosis of fibres also occur in skeletal muscle, and that there is inhibition of the later stages of spermatogenesis. Studies of mice fed the yeast-protein diet plus feeding of wheat germ oil are incomplete.

Correlatives of urinary salt excretion. A. V. WOLF. *Dept. of Physiology and Pharmacology, Albany Med. College, Albany, N. Y.*

The rates of excretion of urinary Na and K were followed for 5 hours in dogs, after intravenous priming with variously concentrated NaCl solutions. On the average, for a given load of salt solution, the disappearance of the load of water and of Na, and the disappearance of the

osmotic distortion is practically a first order process. But there is evidence that the velocity constant of Na increases with the initial distortion. Study of coefficients of correlation between *a*) excretion rates of Na or Na + K and *b*) load of Na or various, calculated osmotic distortions of intracellular and extracellular phases suggests: *1*) the excretion of osmotically effective salts can be described better in terms of osmotic distortions in body fluid compartments than in terms of salt load alone; *2*) the augmentation of K excretion with hypertonic infusions may reflect a compensatory renal activity facilitating the reduction of distortion in body fluid compartments. The manner of formulating the distortion factor which is most closely correlated with salt excretion is under investigation.

Quantitative tests for salivary amylase activity by a new method. E. ALFRED WOLF (introduced by T. K. KRUSE). *Dept. of Biological Sciences, Univ. of Pittsburgh, Pittsburgh, Pa.*

A new method was developed which allows: *1*) the separate collection of the parotid and sublingual-submaxillary (S-S) secretions, and *2*) the timing of the process of digestion of a starch solution by a sample of diluted saliva, without interfering with the process. The reciprocal of the time required for completion of the process (achromatic iodine point), multiplied by 100, is used as the measure of digesting strength. A total of 85 individuals was tested for each kind of saliva: eight adults each 10 times, one adult twice daily 54 times, 76 adolescents each twice. Results: the mean values for adults were: parotid 32.5 units, S-S 5.9 units; for adolescents: females 39.4 and 7.9, males 30.6 and 4.8, negroes 39.3 and 8.6, whites 34.2 and 5.3. The 108 tests on one individual showed identical strength of activity before and after lunch. Addition of mucin-rich S-S saliva, inactivated by heat, to parotid saliva showed no inhibiting effect of mucin. Twice during the testing this person suffered a head cold; the amylase activity was reduced but returned to the normal range after administration of aspirin-codeine for 2–3 days. A linear correlation was found between rate of metabolism per kilogram of body weight, and S-S activity. The tests suggest that strength of salivary amylase activity is a constitutional characteristic of the individual.

I^{131} metabolism by thyroid glands of hypophysectomized C_3H mice. S. H. WOLLMAN AND R. O. SCOW (introduced by ROY HERTZ). *Natl. Cancer Inst. and Natl. Inst. of Arthritis and Metabolic Diseases, Natl. Insts. of Health, Bethesda, Md.*

Four-month-old male C_3H mice were fed a low-iodine pelleted diet in the first experiments. Twenty-four hour I^{131} uptake of thyroids was 18% 2 days post-hypophysectomy, 4% at 6 days, 2% at 12 days, and 20% in shams. Six % of thyroid I^{131} was present in thyroxine fraction 2 days post-hypophysectomy, 3% at 4, and 20% in shams. Thyroid/serum iodide concentration (TSI) ratio after single injection of propyl thiouracil was 75 at 7 days post-hypophysectomy, 125 in normals. In the following studies, 5-months-old C_3H mice were fed since weaning a diet containing 0.25% thiouracil until 7 days pre-operatively and then fed low-iodine diet. Twenty-four hour I^{131} uptake was 54% 2 days post-hypophysectomy, 18% at 6 and 4% at 12 days, compared with 64%, 57% and 45%, respectively, in shams. TSI ratio after single injection of propyl thiouracil was not significantly different from sham value of 145 at 2 and 6 days, but significantly less at 12 days post-hypophysectomy. TSI ratio in unblocked glands, determined with acid soluble I^{131} of thyroid homogenates, was 65–80% lower than in blocked thyroids in both hypophysectomized and normal mice. At 6 days post-hypophysectomy the TSI ratio was normal in unblocked thyroids 1.5 hours after I^{131} administration. At this time bound I^{131} of hypophysectomized mice was 6% that of shams, whereas total uptake was 20% that of shams. Blood I^{131} in hypophysectomized mice was 70% greater than in shams at this time. Unbound I^{131} in thyroids was 50–70% of total gland content in hypophysectomized mice but only 5–10% in shams.

Water and calcium shifts induced in guinea pigs by lethal dose of histamine. LESTER F. WOLTERINK, MARVIN MURRAY* AND W. DOYNE COLLINGS. *Dept. of Physiology and Pharmacology, Michigan State College, East Lansing.*

Terminal quantities of water and Ca^{45} in the tissues of guinea pigs killed with histamine are generally greater than in animals asphyxiated with ether. The increment is small; the major event in histamine death being a massive enlargement of the water space available to I^{131}. In tissues with smooth muscle (uterus, bladder, lung), the entire water content of the tissue becomes available to I^{131}. In pectoral muscle, however, 70% of the total water has still not been entered by I^{131} following histamine. Ca^{45} diffuses beyond the extracellular compartment into the cells where it presumably either exchanges with a certain fraction of the cellular calcium, or is bound to protein or lipid, or both. In ether asphyxiated animals, about half of the total Ca^{45} found in the wet tissue is intracellular (except uterus and liver), if we accept the I^{131} space as the extracellular fluid volume. Following histamine, it is questionable whether the I^{131} space should be regarded as completely 'extracellular'. In pectoral muscle, following histamine, seven-eighths of its Ca^{45} is in a compartment not entered by I^{131} (whereas following ether asphyxia, only half is so distributed) despite doubling of the I^{131} compartment following histamine. Gross immobilization

of Ca^{45} must have occurred in pectoral muscle following histamine. In lung, on the other hand, Ca^{45} was actually lost. (This work was supported in part by the U. S. Atomic Energy Commission.)

Infrared spectra of muscle cells. D. L. Wood and G. B. B. M. Sutherland (introduced by D. E. S. Brown). *Dept. of Physics, Univ. of Michigan, Ann Arbor.*

The infrared spectra of both intact and dehydrated muscle cells have been obtained in the range 1.2μ to 15μ. Various techniques have been employed to do this, including the use of a reflecting microscope. Studies of dehydration and fixation techniques have shown that the spectrum of the intact tissue is changed with standard fixatives, but that freeze drying produces very little change. Experiments with the contractile system of muscle obtained by cold glycerol extraction have so far yielded very little information because of the intense absorption due to glycerol in the region 6–10μ. One of the main experimental difficulties in studying the intact cell in Ringer's solution is that two of the principal bands of muscle protein at 3.0μ and 6.0μ are overlaid by absorption bands due to water in these same regions. Attempts to overcome this difficulty by replacing the water by deuterium oxide have only been partially successful. The principal absorption bands are very similar to those of myosin and actomyosin with the exception of a band near 9.7μ which is quite variable in intensity among muscles from one animal and also between similar muscles from different species. Using polarised radiation, dichroism has been found in a band at 1.95μ in certain cases. The significance of these results will be discussed in relation to current theories of the structure and orientation of protein molecules in muscle.

Simplified calculation of cardiac output by dye method utilizing the oximeter. Earl H. Wood and Homer R. Warner.* *Mayo Fndn., Univ. of Minnesota, and Mayo Clinic, Rochester.*

The technical difficulty of collection and individual analysis of multiple arterial samples in the dye dilution procedure has been eliminated by the development of methods for continuous recording of dye concentration in whole blood. Still remaining, however, is the necessity for tedious semilogarithmic replotting of the curve, extrapolation of the replotted curve to the base line and estimation of the area under this curve to obtain the mean concentration (c) during, and the time (t) required for, initial passage of the dye: factors needed for calculation of cardiac output by the Hamilton formula: $CO = I/ct$. As an empirical simplification, each cuvette oximeter curve was reduced to a triangle by drawing through it three straight lines connecting the points of: initial appearance of the dye, maximal concentration of the dye and the point of intersection of the extrapolated returning deflection with the zero concentration line. The base of this triangle was taken as (t) and one half the altitude as (c). Dye curves recorded from 21 subjects were analyzed by the established semilogarithmic replot method and the triangle method. The triangle method gave values for mean concentration higher (average: 141 $\pm$ 2%) and for passage time smaller (65 $\pm$ 1.5%) than the replot method. Since these were opposing errors, systematic error in cardiac output by the triangle method was small (9 $\pm$ 2%). Standard deviation between triangle and replot values was 9%. Variability, obtained by ear oximetry without blood samples, although large (17%), may not be entirely prohibitive.

Properties of spinal cord elements studied with intracellular ultramicroelectrodes. J. Walter Woodbury* and Harry D. Patton. *Dept. of Physiology and Biophysics, Univ. of Washington School of Medicine, Seattle.*

Ultramicroelectrodes (tip diameter <0.5μ) were introduced into lumbar or sacral spinal segments of decapitate cats. Stimulating and recording electrodes were placed on the dorsal root (DR) and ventral root (VR). Movement of the cord was minimized by three-point fixation bilateral pneumothorax and curarization. Criterion for intracellular penetration of electrode was sudden appearance of a sustained negative resting potential. These measured 40–100 mv. (av. of 21 measurements—69 mv.). Positive monophasic action potentials were elicited by DR and/or VR stimulation. Units responding to VR and DR shocks were designated anterior horn cells; those responding only to DR volleys were arbitrarily termed interneurons. Interneurons characteristically responded repetitively (max. frequency—700 to 1000/sec.) to single DR shocks, the number of spikes being graded by strength of excitatory volley. In some units, spontaneous discharge was accelerated by DR stimulation; in others, inhibited. Latencies of interneuron discharges varied from 1.5 to 5.5 msec, with amplitudes up to 100 mv. The take off of the spike was always sharp with no sign of pre-spike electrical activity. Horn cells were less frequently isolated and differ from interneurons as follows: a) they do not characteristically respond repetitively to single DR volleys, and b) spike is preceded by low amplitude positive potential as described by Eccles.

Effects of some steroids on membrane potentials of single ventricular fibers of frog heart. Lowell A. Woodbury and Hans H. Hecht.* *Depts. of Physiology and Medicine, Univ. of Utah, College of Medicine, Salt Lake City.*

Because digitoxin and other cardiac glycosides caused such striking effects on the membrane rest-

ing and action potentials of single ventricular fibers of the frog heart, work was extended to steroids and of necessity to the liquid vehicles in which they were suspended. Preliminary results were as follows: Desoxycorticosterone acetate in doses of 250–500 mg/kg. given 24–48 hours before examination caused no consistent observable changes. The intravenous injection of the glycoside of this steroid in doses of 25 mg/kg. was often followed by transitory decreases in the membrane potential, the overshoot potential and the first phase of recovery but the results were not always consistent. Cortisone and Compound F in doses up to 30 mg/kg. caused no consistent changes within two hours that could be attributed specifically to these steroids. Cholesterol in a dose of 7.4 mg/kg. resulted in a bizarre action potential with a transitory increase in the rate of the first and second phases of recovery and a decrease in the third phase. Negative after potentials of large magnitude (10–12 mv.) appeared following the cholesterol. Recovery from the effects was complete in about an hour. Na-androsterone sulfate at a dose of 75 mg/kg., i.v., caused a 40% decrease in the duration of the action potential and an increased rate of recovery of phases 1 and 2. The vehicle (Merck aqueous vehicle no. 1) in which some of the steroids were suspended when given in doses of 1.5–2.5 ml/kg. resulted in a very marked decrease in the rate of the first phase of recovery, an increase in the overshoot potential and an increase in the ratio of the ventricular contraction duration to the action potential duration.

Patterns of localization in somatic efferent and afferent systems of the cerebral cortex of the chimpanzee. C. WOOLSEY, J. BARNARD,* R. BUTLER,* G. CRANDALL,* J. FAY,* R. OSTENSO,* P. SETTLAGE* AND A. TRAVIS.* *Dept. of Physiology, Med. School, Univ. of Wisconsin, Madison.*

Two chimpanzees were maintained under Nembutal anesthesia for 5 and 7 days, respectively, while studies were made continuously day and night through rotation of personnel. Of 6 problems studied on each of these animals, results will be reported on the following 3: *1*) The precentral motor area, including most of its buried portions, was explored in 2-mm. steps with 60-cycle a. c. stimuli of 3-second duration and of threshold intensity. *2*) The supplementary motor area on the medial aspect of the hemisphere was located and partly defined. *3*) The postcentral tactile area was mapped with the evoked potential method. Results of these 3 studies will be presented as figurine charts and the relations found in the chimpanzee will be compared with those previously reported in this way for other primate and subprimate forms.

Use of the stereoencephalotome in man for subcortical stimulation, recording of potentials and production of lesions (Motion picture). H. T. WYCIS* AND E. A. SPIEGEL. *Depts. of Exptl. Neurology and Neurosurgery, Temple Univ. School of Medicine and Hosp., Philadelphia, Pa.*

The stereoencephalotome is an application of Horsley-Clarke's stereotaxic principle to the human brain. The motion picture shows the preoperative x-ray studies, the calculation of the electrode position in relation to intracerebral reference points (pineal gland, posterior commissure), the insertion of stimulating and recording electrodes, the recording of subcortical potentials, the production of electrolytic subcortical lesions, and postoperative controls of the position of the lesion. The use of the apparatus for physiologic studies during operation and the range of error of the method are discussed.

Measurement of dead space during exercise and at rest. ALLEN C. YOUNG (introduced by H. S. BENNETT). *Dept. of Physiology and Biophysics, Univ. of Washington School of Medicine, Seattle.*

The dead space was measured by using a rapid CO_2 analyzer. Measurements on subjects exercising (7.5 mph, 7.5% grade) and doing voluntary expiration from various initial lung volumes showed a variation of dead space of approximately two to one. If Bohr's equation and end alveolar samples had been used in the calculation of dead space very much larger changes would have been falsely indicated.

Respiration and anaerobic glycolysis of developing limb buds of chick embryos. WASLEY D. YUSHOK* AND WIKTOR W. NOWINSKI. *Tissue Metabolism Research Lab. and Dept. of Biochemistry, Univ. of Texas Med. Branch, Galveston.*

The oxygen uptake and anaerobic glycolysis of the developing wings and legs of 5- to 10-day embryos were investigated in order to determine which enzyme system is more closely related to growth. A cross of White Leghorns and New Hampshire Reds was used. The results, expressed in microliters per hour of O_2 uptake or of CO_2 evolution, were calculated on the following bases: total appendage; 1 mg. dry weight; and 100 micrograms DNA, which is equivalent to approximately 15 million cells. Oxygen uptake of wings, calculated on these bases, was not significantly different from that of legs. On the DNA basis, the anaerobic glycolysis of the legs of 6- and 7-day embryos was significantly higher than that of the slower growing wings. The values (mean and standard error) for 7-day wings and legs were 20.4 ± 0.40 and 23.6 ± 0.55 respectively. However, on the dry matter basis, there were no statistical differences between wings and legs of the investigated stages. Comparions of the instantaneous rates of growth and of metabolic processes revealed the following: When the rate of DNA accumulation in legs was increasing, the rate of O_2 uptake was decreasing

and vice versa. However, the rate of CO_2 production of legs was running parallel to that of DNA accumulation from the 5th to the 7th day. In the wings, the relation of oxygen uptake and of DNA was similar to that of the legs.

Accelerating effect of adrenalectomy on regrowth of hair in the rat considered in relation to pituitary histology. ISOLDE T. ZECKWER. *Dept. of Pathology, Univ. of Pennsylvania Medical School, Philadelphia.*

Four groups of rats were studied: *1*) thyroidectomized, *2*) thyroidectomized and adrenalectomized, *3*) adrenalectomized, *4*) intact. Adrenalectomy was performed 5 to 17 weeks after thyroidectomy. Adrenalectomized rats received 1% NaCl drinking fluid. Hair was shaved by electric clippers from the back and sides of all rats, and the regrowth of hair photographed. Thyroidectomized rats showed large bald areas; rats with combined thyroidectomy and adrenalectomy showed rapid diffuse regrowth, nearly as marked as in adrenalectomized with thyroids intact, whose growth was much more rapid than in the intact rats. This proved that thyroidectomy, which caused loss of acidophils from the pituitary and stunting of somatic growth, did not prevent the accelerating effect of adrenalectomy on hair growth. A number of parabiotic rats were studied in which one partner was adrenalectomized. The adrenalectomized partner showed as rapid diffuse regrowth of hair as did single adrenalectomized rats. The intact partner in parabiosis with the adrenalectomized showed the slower patterns of regrowth characteristic of a single intact rat. The adrenalectomized partner, therefore, did not transfer to the intact parabiont any substance that stimulated hair growth. This suggests that adrenalectomy does not cause any increase in an accelerating substance, but occasions the removal of an inhibitory substance, and this removal is not modified by loss of pituitary acidophils consequent upon thyroidectomy. Regrowth of hair does not parallel somatic growth and does not depend on the presence of pituitary acidophils.

Measurement of limb blood flow in man by constant infusion of T-1824. K. L. ZIERLER, R. ANDRES,* H. M. ANDERSON,* W. STAINSBY* AND J. L. LILIENTHAL, JR. *Depts. of Environmental Medicine and Medicine, Johns Hopkins Univ., Baltimore, Md.*

T-1824 is injected into the brachial artery at constant rate by a motor-driven syringe. Venous blood is collected continuously from a catheter in an ipselateral antecubital vein. From the known rate of infusion and the concentration of dye in venous plasma, the rate of plasma flow can be calculated by the Stewart principle. Theory assumes uniform concentration of dye throughout the vascular bed under study, but this is not attained if infusion is performed through an ordinary hypodermic needle or at very slow rates, owing to uneven distribution of infusate in a laminar-flow system. However, when the infusion is jet-sprayed against the blood stream through a needle of approximately 1 ml. internal diameter uniform concentration is attained, as demonstrated in a mechanical flow model, in the femoral artery of the dog, and in the forearm in man. If after equilibrium is attained, the injection is halted, then the concentration of T-1824 in the venous effluent (corrected for recirculation) vanishes with time. The area under the falling curve at the determined rate of flow provides a measure of the vascular volume. (Aided by contract with Office of Naval Research, NR 113-241.)

Effect of Dibenamine on humoral and vascular changes in dogs subjected to hemorrhagic shock. BENJAMIN W. ZWEIFACH, SILVIO BAEZ AND EPHRAIM SHORR. *Dept. of Medicine, Cornell Univ. Med. College and New York Hosp., New York City.*

Conventional hemodynamic measurements have not disclosed the mechanisms by which Dibenamine, an adrenolytic and sympatholytic drug, protects the peripheral circulation against the lethal effects of hemorrhagic or traumatic shock. The present studies with Dibenamine derivative, S.K.F. 688A, in the dog have utilized criteria of peripheral vascular function obtained by direct visualization of the terminal vascular bed in the omentum, and by assay for blood-borne vasoactive principles. Dogs, given 2 mg/kg. 688A, readily withstood an otherwise lethal graded hemorrhage and recovered following administration of the blood withdrawn. Although Dibenamine in this dose range is adrenolytic, the muscular vessels in the capillary bed before hemorrhage continued to respond to normally effective concentrations of epinephrine applied topically to the omentum. Furthermore, Dibenamine did not interfere with the development of hyperreactivity nor with the augmentation of vasomotion in the terminal vascular bed, characteristic of the compensatory response to hemorrhage. The outstanding differences in the omentum were a blunting of the vasoconstriction usually observed in the arterioles and the persistence of a remarkably adequate circulation through the capillary bed at pressures as low as 25 mm. Hg. Vascular decompensation did not develop following a period of hemorrhagic hypotension which produced hyporeactivity and irreversibility in controls. Although the elaboration of VEM by the kidney during the compensatory stage was not affected, VDM failed to appear, even after 3 to 4 hours of drastic hypotension, and the liver was found to have preserved the integrity of its VDM inactivation mechanisms.

AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS

Forty-third Annual Meeting

NEW YORK CITY, APRIL 14–18, 1952

(An asterisk following an author's name indicates "by invitation")*

Purification and properties of prolidase from erythrocytes. ELIJAH ADAMS* AND EMIL L. SMITH. *Lab. for Study of Hereditary and Metabolic Disorders, Univ. of Utah, Salt Lake City.*

Individual peptidases which hydrolyze the substrates, glycyl-L-proline (GP), L-leucinamide (LA), glycylglycine (GG) and triglycine (GGG), were found in erythrocytes of several species. Levels for these enzymes are relatively constant in individual human hemolysates and show metal activation characteristics for the hydrolysis of GP, LA and GG similar to comparable activities in other tissues. Hemolysates of nucleated avian erythrocytes are similar in levels of activity to mammalian hemolysates. Prolidase (hydrolysis of GP) was partially purified from horse erythrocytes by ammonium sulfate fractionation after removing hemoglobin by the Tsuchihashi procedure. Preparations were 100–400 times purer than crude hemolysates and comparable to the best obtained from other tissues. Erythrocyte prolidase prepared as described is virtually free of contaminating tripeptidase, aminopeptidase and a variety of dipeptidase activities. Similarity of prolidase from erythrocytes and from other tissue sources was indicated by: optimal hydrolysis of GP at pH 7.8, 10- to 30-fold activation by incubation with 0.02 *M* Mn^{++} but not with other metals studied, inhibition by pyrophosphate and versenate, and by the relative proteolytic coefficients for GP and the less sensitive substrates, glycylhydroxy-L-proline and β-alanyl-L-proline. Studies of specificity indicated the requirement of free α-NH_2 and α-COOH groups for significant hydrolysis. No proteinase activity was detected on prolonged incubation with horse hemoglobin or human serum albumin.

Selection of biochemically deficient mutants of *E. coli*. EDWARD A. ADELBERG AND JOHN W. MYERS (introduced by M. DOUDOROFF). *Dept. of Bacteriology, Univ. of California, Berkeley.*

The use of penicillin to select for auxotrophic mutants induced in a wild-type population of *E. coli* has 3 drawbacks as currently employed: mutant survival is variable and frequently very low, wild-type survival is often high, and the use of an intermediate cultivation period in liquid medium results in an unknown number of replicates of each original mutant appearing as separate colonies on the final plates. This means that when a biochemist attempts to collect mutant strains blocked at several different steps in a given biosynthetic pathway, many thousand wild-type colonies may have to be isolated, screened and discarded; and even when several desired types are found they may all be identical progeny of one original mutant cell. A new technique has been devised which eliminates these difficulties and greatly simplifies the manipulations. Wild-type cells of *E. coli* (ATCC 9637) are irradiated with ultra-violet and about 3×10^3 survivors plated in one petri dish in minimal agar. To the plate are successively added, with appropriate intervening periods of incubation, layers of minimal agar containing penicillin, penicillinase, and finally growth factors. Between 70 and 80 colonies eventually appear, 95% of which are stable biochemical mutants, each representing a unique mutation event. In one experiment 20% of these were found to respond to hydrolyzed nucleic acid, 10% to a vitamin mixture, and 70% to vitamin-free hydrolyzed casein. Virtually complete recovery and selection of mutants have been obtained without exception in 30 or more experiments. (Supported in part by ONR contract.)

Biochemical effects of specific amino acid deficiencies in man. ANTHONY A. ALBANESE AND REGINALD A. HIGGONS.* *Nutritional Research Lab., St. Luke's Hosp., New York City.*

Previous publications by others and the authors have shown that diets poor or lacking in each of the indispensable amino acids caused abnormal N-retention in infants and negative N-balances in young adults. In an attempt to establish criteria for the detection of spontaneous deficiencies of specific amino acid in man the experimentally induced N losses were characterized by measurements of various N and S constituents of the urine and blood protein levels. In infants a temporary lack of tryptophane, isoleucine, lysine and the sulfur amino acids produced such changes in output of urea, ammonia, organic acids and various amino acids as to permit easy identification of each deficiency. Variations in the levels of various blood

proteins were also specific. The N-losses of adults maintained on diets poor in tryptophane, histidine, lysine and the sulfur amino acids could not be so characterized. The biochemical significance and some applications of these findings will be discussed.

Thiosulfates and methionine metabolism in man. ANTHONY A. ALBANESE, BETTY L. VESTAL* AND LOUKIA STEPHANSON.* *Nutritional Research Lab., St. Luke's Hosp., New York City.*

Recent studies have shown that in descending order, thiocyanate, thiosulfate, sulfite and hydrosulfite ions interfere with the oxidimetric determination of methionine in biological products. Of these interferants only the thiosulfate ion requires consideration when the measurement is made in human urine. The average daily thiosulfate sulfur output, determined iodometrically, was 35.5 mg. for children and young adults, (7–26 yrs.) and 29.1 mg. for older adults (68–80 yrs.) This amount of thiosulfate sulfur introduces a positive error in this measurement of methionine which is equivalent roughly to 20% of the daily methionine output of children and young and old adults. Oral administration of 1.5–3.0 gm. of DL-methionine to children and young adults led to an increased output of methionine, cystine and inorganic S, but failed to increase the thiosulfate titre above normal levels. Similar tests with L-methionine also failed to change the thiosulfate titre from the norm. These findings suggest that, unlike in other species, the thiosulfate ion is not a primary or immediate metabolite in the human metabolism of methionine.

Stability of human plasma prothrombin fractions. BENJAMIN ALEXANDER AND GRETA LANDWEHR.* *Yamins Research Lab., Beth Israel Hosp., and Dept. of Medicine, Harvard Med. School, Boston, Mass.*

Several methods have been employed to separate prothrombin from liquid or lyophilized human plasma: *a*) direct adsorption of oxalated, or resin-decalcified, plasma with BaSO4 and elution with citrate; *b*) adsorption-elution of citrated plasma after prior removal of calcium and citrate by ion-exchange resins; *c*) initial precipitation of the euglobulins at low ionic strength and pH 5.1 followed by BaSO4 adsorption and citrate elution of the euglobulin fraction; *d*) same as *c*) but elution with phosphate instead of citrate; and *e*) subfractionation of the euglobulins with ammonium sulphate. In contrast to preparations from fresh plasma, those from aged or lyophilized plasma exhibit marked instability of the prothrombin activity, substantial losses or total inactivation occurring within 24–48 hours during handling. Progressively less thrombin can be evolved in the 2-stage system despite supplements of known conversion factors. The losses are not reflected in thrombin formation except with procedure *d* where phosphate instead of citrate is used as eluant. The latter observations are in accord with Milstone's, on bovine prothrombin similarly derived, who attributed the phenomenon to prothrombin conversion by thrombokinase in the fractions. Experiments are in progress to ascertain whether the lability of our fractions is similarly referable to thrombokinase formation during the aging of plasma. Precipitation of the prothrombin with the euglobulins as the first step in the procedure has notable advantages: *1*) interference by citrate anticoagulant with prothrombin adsorption by BaSO4 is obviated; *2*) Spca and its precursor are not precipitated with the prothrombin, and can be subsequently separated from the non-precipitated fraction.

Distribution of exogenous cholesterol in tissues of rat. ROSLYN B. ALFIN-SLATER AND LESLIE I. RICE (introduced by HARRY J. DEUEL, JR.). *Dept. of Biochemistry and Nutrition, Univ. of Southern California, School of Medicine, Los Angeles.*

The incorporation of exogenous cholesterol into the tissues of normal rats was studied by feeding a diet containing 1% of deuterium-labeled cholesterol for a period of 8 days. Food consumption was measured and urine and feces were collected over the experimental period. At the end of 8 days the animals were sacrificed and total cholesterol and deuterium content of cholesterol were measured in a series of tissues. There was a significant increase in cholesterol in the liver, adrenal, and intestine. Although the mg. cholesterol/gm. of spleen did not increase significantly, the spleens of the cholesterol-fed animals were somewhat enlarged and therefore the total cholesterol content was greater than in the control group. Deuterium-labeled cholesterol was found in all the tissues examined, although only a negligible amount occurred in the brain. The deuterium concentration was highest in the cholesterol of the feces and was identical with the deuterium concentration of the cholesterol fed. The next highest deuterium concentration was found in the liver, lung, spleen and adrenal, and decreased respectively in red blood cells, plasma, intestine, carcass and kidney. The deuterium concentration in the body fluids was negligible. This indicates the transfer of cholesterol into tissues other than liver and into tissues where total cholesterol levels have not been changed. (Supported by a grant from the U. S. Public Health Service.)

Serum tributyrinase III. Effect of pH and ionic strength on enzyme activity. C. ALPER AND P. P. POLAKOFF, JR. (introduced by M. J. BOYD).

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A study of the effect of pH on the activity of serum tributyrinase demonstrates the presence of 2 peaks of enzyme activity. Emulsions of tributyrin (ALPER AND POLAKOFF, *Federation Proc.* 10: 155, 1951) are prepared in veronal, phosphate, glycine, citrate and borate buffers of identical ionic strength. All determinations are performed in the presence of Ca^{++} with the exception of phosphate buffer. The pH optima of serum tributyrinase in each of the buffers are respectively, veronal, 6.5 and 8.5; phosphate, 6.0 and 7.5; glycine, 6.0 and 9.5; citrate, 7.0 and 8.5; and borate, 6.5 and 8.5. The tributyrinase activities/ml. of serum at the alkaline and acid peaks are respectively: veronal, 1.17 and 0.97 units; borate, 1.12 and 0.81 units; citrate, 0.83 and 1.01 units; citrate without Ca^{++}, 0.60 and 0.60 units; glycine, 0.82 and 0.75 units; and phosphate, 0.78 and 0.67 units. In every buffer with the exception of citrate the enzyme activity at the alkaline peak exceeds that at the acid peak. A 2-, 4-, and 6-fold increase in the ionic strength of the veronal buffer results in greatly increased tributyrinase activity/ml. of serum and in a progressive shift of the alkaline and acid pH optima to the acid side. The serum tributyrinase activity in veronal buffer at 6 times the original ionic strength is increased to 1.51 units at a pH optimum of 7.5 and to 1.50 units at a pH optimum of 6.0.

Hypervitaminosis A induced by crystalline vitamin A acetate. STANLEY R. AMES, WILLIAM J. SWANSON,* HUGH A. RISLEY* AND PHILIP L. HARRIS. *Research Labs., Distillation Products Industries, Div. of Eastman Hodak Co., Rochester, N. Y.*

Hypervitaminosis A in the albino rat was investigated, employing cottonseed oil solutions of crystalline vitamin A acetate. Single dose feeding tests indicated an LD_{50} of about 1 million units/100 gm. body weight. Results of 28 day, semi-chronic tests showed no deaths at a level of 15,000 units/100 gm/day and an LD_{50} at 28 days of 20,000–30,000 units/100 gm/day. When animals were supplemented daily over a long period (9–12 months), about ½ the animals died at levels of 20,000 units/animal/day and all died at 30,000 units. Depression of the growth curve proved to be a very sensitive measure of vitamin A toxicity. Supplements as high as 10,000 units/100 gm/day showed normal growth but levels of 15,000–20,000 units/100 gm/day caused marked depression. Organ weights at the end of the experimental periods were essentially normal with the exception of a marked hypertrophy of the adrenals. Liver storage of vitamin A at the 28th day was over 50% of ingested dose up to 12,000 units/100 gm/day. Apparently the liver reaches maximum vitamin A storage at about 50,000 units/gm. of fresh liver tissue. At this level of storage the extractable liver oil has a potency of about 1 million units/gm. Kidney storage of vitamin A was proportional to the dose at all levels. The breaking strength of the excised leg bones decreased with increasing dosage and closely paralleled depression of growth as a measure of vitamin A toxicity.

Changes in blood cholesterol and lipoproteins during experimental overnutrition. JOSEPH T. ANDERSEN,* ADRIAN LAWLER,* MAX LOWEN* AND ANCEL KEYS. *Lab. of Physiological Hygiene, Univ. of Minnesota, Minneapolis, and Mental Health Research Lab., State of Minnesota, Hastings.*

Twenty middle aged male schizophrenic patients were encouraged to eat as much as they would of a well balanced high calorie diet for 20 weeks. The body weight grains were from 2–22 kg. Three men gained more than 20 kg. There was a significant correlation between increase in total serum cholesterol concentration and body weight gain; the coefficient of correlation was 0.47 and the probability of this value occurring by chance was 0.04. The slope of the regression line indicated that for each kg. increase in weight the serum cholesterol rise was 2.2 mg/100 ml. At the middle and at the end of the fattening period the concentration of the Gofman S_f 10–20 lipoprotein in the serum of 12 men was determined. This lipoprotein consists of particles which when suspended in a medium of specific gravity 1.063 in the ultracentrifuge undergo flotation at rates between 10 and 20 Svedberg units. The relationship between increase in Gofman lipoprotein in the last half of the fattening period and weight gain in the same interval gave a correlation coefficient of 0.48 and a probability of chance occurrence of 0.11. The lower level of significance for the lipoprotein than for total serum cholesterol is probably due in part to the smaller number of observations. The relation of serum cholesterol and Gofman lipoprotein to fatness in groups of subjects who have maintained their weight over a period of years will be discussed.

Transcapillary movement of sodium and calcium. W. D. ARMSTRONG, JOHN A. JOHNSON,* LEON SINGER* AND ROGER LIENKE.* *Dept. of Physiological Chemistry, Univ. of Minnesota, Minneapolis.*

Arterial plasma concentrations of Ca^{45} have been determined in 6 dogs between 1 and 150 minutes after the intravenous injection of Ca^{45} in aqueous solutions and in plasma. Two animals received simultaneously Na^{22} and Ca^{45}, and arterial plasma concentrations of each isotope were determined at

intervals to 340 minutes. The data have been fitted to equations in 3 exponential terms (Na^{22}) or 4 exponential terms (Ca^{45}). The apparent rates of transcapillary movement of sodium and calcium calculated from these equations are approximately equal. These calculations indicate, in different animals, that 30–60% of plasma sodium and calcium are turned over per minute. The concentration of Ca^{45} in plasma declines more rapidly than that of Na^{22} due to exchange of Ca^{45} for Ca^{40} of bone resulting in a reduced net return of Ca^{45} from extravascular fluids to plasma. Evidence has been obtained that bone calcium is not equally exchangeable for that of extravascular fluids and that no longer than 3 minutes is required for some calcium to move from plasma to bone. Calcium binding by plasma proteins does not appear to interfere with transcapillary movement of calcium.

Metabolic studies of radiotestosterone. JAMES ASHMORE* AND EDWARD A. DOISY. *Dept. of Biochemistry, St. Louis Univ. School of Medicine, St. Louis, Mo.*

Testosterone-4-C^{14} was prepared by the method of Turner. The radioactivity of this labeled steroid was 3.2×10^5 c/m/mg. Its metabolism was studied by determining the excretion of C^{14} in urine, feces, and expired air after the intragastric or intramuscular administration of 0.2 mg. of testosterone-4-C^{14} to normal male rats. No C^{14} was found in the expired air. Slightly more radioactivity was found in feces than in urine. Two hundred μg. of radiotestosterone was administered by stomach tube to male rats with bile fistulas or with ligated bile ducts. The absence of significant amounts of fecal radioactivity during the 4-day experimental period indicated almost complete absorption. The bile was found to be the major pathway of excretion in the bile fistula animals; in the duct ligated rats, the major amount of C^{14} appeared in the urine.

Comparison of rates of reaction of bound and free diphosphopyridine nucleotide. LAZARUS ASTRACHAN (introduced by NATHAN O. KAPLAN). *McCollum-Pratt Inst., Johns Hopkins Univ., Baltimore, Md.*

Taylor *et al.* (*J. Biol. Chem.* 173: 619, 1948) demonstrated that triosephosphate dehydrogenase from rabbit muscle crystallizes with DPN firmly attached to the protein. We have made an attempt to determine which functional groups of DPN are involved in its attachment to triosephosphate dehydrogenase by comparing the rates of reaction of bound and free DPN with various enzymes. Bound DPN reacted at ½ the rate of free DPN with *Neurospora* DPNase, intestinal phosphatase and snake venom nucleotide pyrophosphatase. Bound DPN was deaminated by takadiastase deaminase at ¼ the rate of free DPN, and was reduced with yeast alcohol dehydrogenase at ⅛ the rate of free DPN. A sensitive and specific assay method was developed to follow the degradation of DPN by either phosphatase, deaminase, or pyrophosphatase action. The products of these reactions have the same fluorescence as DPN in 5N alkali, but the contribution to the fluorescence total by DPN was removed specifically by *Neurospora* DPNase. In this manner the degradation of DPN and formation of product could be measured by an increase in fluorescence. The method was developed in order to take advantage of the sensitivity of the alkaline fluorescence assay for DPN (KAPLAN *et al. J. Biol. Chem.* 191: 461, 1951).

Oxidative metabolism of hexose phosphates by higher plants. BERNARD AXELROD AND ROBERT S. BANDURSKI.* *U. S. Dept. of Agriculture, Albany, Calif., and Kerckhoff Biological Lab., California Inst. of Technology, Pasadena.*

Studies with soluble extracts of acetone powders of pea and spinach leaves show the operation of the Warburg-Lipmann-Dickens scheme for the oxidative metabolism of glucose-6-phosphate. When the extract was incubated with glucose-6-phosphate, TPN was reduced, and a Meijbaum-reactive substance was formed. When the extract acted on C^{14}-labelled 6-phosphogluconic acid, in the presence of TPN, $C^{14}O_2$ was produced and radioactive ribose-5-phosphate was identified chromatographically as a reaction product. The regeneration of the catalytic amount of TPN present was accomplished by the addition of GSSG, since GSSG-reductase is present in the extract. Reoxidation of $TPNH_2$ may also be carried out by 2,6 dichlorophenolindophenol or may be coupled to O_2 by the addition of methylene blue, since diaphorase occurs in the extract. Both fructose-6-phosphate and fructose diphosphate formed Meijbaum-reactive material upon incubation with the extract. The utilization of the first substrate can be explained by the known presence of phosphohexose isomerase, while that of the second is perhaps due to its conversion to fructose-6-phosphate by phosphatase. Incubation of ribose-5-phosphate with the extract resulted in a much greater disappearance of Meijbaum-reactive material than could be accounted for on the basis of phosphoribose-phosphoribulose isomerization; the presence of TPN was not required. Pentose disappearance was accompanied by the liberation of inorganic phosphate, the latter reaction occurring at a much slower rate, on an equivalent basis.

Paper partition chromatography of progesterone and related compounds. LEONARD R. AXELROD

(introduced by HAROLD C. HODGE). *Dept. of Radiation Biology, Univ. of Rochester, School of Medicine and Dentistry, Rochester, N. Y.*

Techniques of paper partition chromatography using non-aqueous solvent systems have been developed for the quantitative separation of progesterone and its related steroid compounds. Quantities ranging from 8–30 μg. can be detected in developed chromatogram strips by several tests including the use of concentrated and fuming (15%) sulphuric acid. Preliminary studies of urines from normal and pregnant females have revealed, as previously reported with other techniques, the presence of pregnanediol, progesterone, and allopregnane-3 β-ol, 20-one, as well as other compounds included within this series.

Malate system of *Trypanosoma cruzi*. H. D. BAERNSTEIN. *Natl. Insts. of Health, Bethesda, Md.*

In homogenates of the culture form of *Trypanosoma cruzi*, the system which oxidizes malate is linked to DPN and gives oxalacetate as product. The reaction is reversible and the equilibrium favors formation of malate. Oxalacetate prevents malate oxidation at pH 7–8 but not at 8–10. The optimum pH is 9.5 without trapping agents for oxalacetate. Michaelis constants are 1.6×10^{-2}M malate and 3×10^{-3}M oxalacetate. The system may be linked to endogenous transaminase since glutamate relieves the block due to oxalacetate in the forward reaction, and aspartate + αketoglutarate may be substituted for oxalacetate in the reverse reaction. Fumarate is not effective when substituted for malate indicating the absence of fumarase. Incubation of DPN with the homogenate shows no splitting of the nicotinamide-ribose link, indicating the absence of DPNase. Iodoacetate (6×10^{-3}M) inhibits 90% indicating functional sulfhydryl groups. However, the enzyme is stable for over a month when stored in air at 5°C. Specific activity for malate oxidation is 1824 units/mg. N and for oxalacetate reduction is 3030 units. One unit is defined as a change of 0.01 in $\log \frac{I^\circ}{I}$ per minute at 340 mμ in a 1.0 cm. cell at 25°C. These activities are equivalent to QO_2 values of 210 and 348 respectively. The total QO_2 of intact organisms metabolizing glucose is 36.2.

Free amino acids in yeast under various conditions. WILLIAM BAIR AND GEORGE ROUSER (introduced by ROBERT WITTER). *Depts. of Radiation Biology and Biochemistry, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Extracts were made and chromatograms prepared as previously described (*Federation Proc.* 10: 240, 1951). Fleischman's bakers yeast was used. The uncultured cake contained large amounts of alanine and glutamic acid; medium amounts of γ-amino butyric acid, glutamine, valine + methionine, and an unknown compound (Unknown 2 R_f values in phenol 0.52, collidine 0.22); small amounts of asparagine, glutathione, glycine, leucine(s) proline, serine, and 2 unknowns (1 and 3, R_f values phenol 0.60, 0.62; collidine 0.14, 0.27); and traces of aspartic acid, lysine, phenylalanine, threonine and tyrosine. Cultures of 18–24 hours in Spiegleman's medium showed increased lysine; appearance of β-alanine, taurine and ornithine; decrease of γ-amino butyric acid and unknowns 2 and 3; and absence of glutamine, proline and unknown 1. The concentration of amino compounds in the medium was much lower than in the cells. Yeast cultured in Spiegleman's medium and then starved by bubbling air through the suspension medium for 48 hours showed a qualitative pattern like that of the unstarved cultured yeast with all compounds greatly decreased in amount. A double spot observed for glutamic acid in collidine may have been due to a compound moving almost to the same position that gives a yellow fluorescent color with 1/1 acetone and 10% sodium hydroxide.

Utilization of some purine ribose derivatives by *Lactobacillus casei*. M. EARL BALIS AND GERTRUDE B. ELION (introduced by GEORGE H. HITCHINGS). *Labs. of the Sloan-Kettering Inst. for Cancer Research, New York City, and Wellcome Research Labs., Tuckahoe, N. Y.*

Comparison of the results of the utilization of ribosides and ribotides with those from the corresponding free purines has led to some information about the pathways involved in the incorporation of purines into nucleic acids. The incorporations of ribosides of adenine, guanine, hypoxanthine, 2,6-diaminopurine and isoguanine, labeled with C^{14} in the purine ring, were studied in a folic acid-containing medium. The individual purines from the PNA were separated by paper chromatography and their activities were determined. Adenosine was converted preferentially into PNA-adenine; guanosine and diaminopurine riboside were converted preferentially into PNA-guanine. Inosine and crotonoside were less efficiently utilized than the above three and the incorporation into the two PNA purines was essentially equal. The pattern of interconversion of these purine derivatives was different from that of the corresponding free bases, which suggests that their utilization is not via the free bases. Also since guanosine and adenosine are less efficiently used than guanine and adenine the free bases are probably not first converted to the ribosides. The utilization of the *b* isomers of adenylic and guanylic acids is much more extensive than that of the *a* isomers, and the extent of utilization would be compatible with the assumption that the *b* nucleo-

tide is one of the first products derived from adenine.

Effect of Roentgen irradiation on tissue specific cathepsins and their inhibitors. JOHN C. BALLIN* AND ROBERT N. FEINSTEIN. *USAF Radiation Lab. and Depts. of Pharmacology and Biochemistry, Univ. of Chicago, Chicago, Ill.*

The Cathepsin II, III, and IV activities of mouse and rat kidney, intestine, and liver were measured in normal animals and those receiving LD_{50} doses of x-irradiation. The x-rays caused an increase in activity of Cathepsin II in kidney, intestine, and liver, of Cathepsin III in intestine, and of Cathepsin IV in kidney. Because ionizing radiation generally exerts a catabolic rather than an anabolic effect, the destruction of a proteolytic inhibitor was suspected. Chloroform extraction of rat kidney caused an increase in the Cathepsin IV activity. Differential centrifugation separated an inhibitor in an assayable form. Rat and rabbit plasma were shown to contain a Cathepsin IV-like enzyme, while a 4% solution of laked blood cells caused almost complete inhibition of rat kidney Cathepsin IV. Furthermore, while laked blood cells from normal animals caused an 80% inhibition of standard enzyme activity, identically prepared cells from x-rayed animals resulted in no loss of activity.

Endocrine regulation of the rate of protein synthesis and size of the nitrogen pool. PAUL D. BARTLETT. *Edsel B. Ford Inst. for Med. Research, Henry Ford Hosp., Detroit, Mich.*

Utilizing technical procedures and mathematical treatment described by Sprinson and Rittenberg (*J. Biol. Chem.* 180: 715, 1949) rates of protein synthesis and size of the nitrogen pool have been quantitatively evaluated in adult female dogs during nitrogen balance and nitrogen storage experiments. In 3 dogs rates of protein synthesis expressed in gm. N synthesized into protein/24 hours/kg. body weight were found to be respectively 0.49, 0.56, and 0.82 during nitrogen balance experiments. Values for parallel determinations of the nitrogen pool were respectively 0.82, 0.91, and 0.95 gm. N/kg. body weight. The respective rates of protein synthesis were increased to 0.86, 0.87, and 1.01, and the size of the nitrogen pool to 1.30, 1.20, and 1.38 gm. N/kg. body weight during nitrogen storage induced with anterior pituitary growth hormone. Induction of smaller amounts of nitrogen storage with testosterone propionate in the first 2 animals resulted in rates of protein synthesis of 0.65 and 0.79 respectively and values of 0.82 and 0.81 for the size of the nitrogen pool. The capacity of growth hormone to produce marked increases in the size of the nitrogen pool is particularly striking, and as previously suggested (*J. Biol. Chem.* 187: 253, 1950) may be indicative of the mechanism by which this hormone regulates intracellular synthesis of protein.

Behavior of thyroid toward elements of seventh periodic group. III. Technetium. EMIL J. BAUMANN, NORMA Z. SEARLE,* A. AARON YALOW,* EDWARD SIEGEL* AND S. M. SEIDLIN.* *Montefiore Hosp., New York City.*

In continuation of our investigation of the filtration of seventh group ions by the thyroid, we report on the distribution of Tc in that organ and in other tissues, following parenteral administration of about 0.1 mg. to hooded rats. Tc^{99} (9×10^5 year half-life; 0.32 Mev maximum beta ray energy) was estimated in tissues by digestion with concentrated H_2SO_4 and 30% H_2O_2 at about 150°C. Tc was precipitated as the sulfide on Re_2S_7 from 4 M H_2SO_4 at 100°C. with $Na_2S_2O_3$, digested on a water bath for several hours, allowed to stand at 5°C. overnight, filtered on a fritted glass crucible and its activity measured with a Bernstein-Ballentine proportional flow counter (*Rev. Sci. Instr.* 20: 347, 1949). As with the other seventh group elements tested by us and by others (Cl Br, I, Mn, Re, At), we find that Tc is preferentially filtered by the thyroid. Two hours after injection, the thyroids contained 15–24% of the injected dose/gm., which is 17–32 times the blood concentration. The total uptake in the thyroid was 0.3–1.0% of the administered Tc. The concentration in skin and kidney almost equalled that in blood, while in other tissues it was only 4% (brain) to 60% (liver) as much. The 2 hour urinary excretion was 5–10% of the injected dose. (Aided by grants from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.)

Glucose oxidase in determination of glucose in biological material. WILLIAM S. BECK (introduced by SIDNEY ROBERTS). *School of Medicine, Univ. of California at Los Angeles.*

A glucose oxidase, prepared from *Aspergillus niger* media (courtesy E. M. Weber, Chas. Pfizer & Co.) is routinely used in this laboratory for glucose determinations. Keilin and Hartree, using an enzyme from *Penicillium notatum* (*Biochem. J.* 42: 230, 1948) found the complete and stoichiometric oxidation of glucose permitted its manometric measurement. Catalase was usually added to ensure breakdown of liberated hydrogen peroxide. The present enzyme preparation appears to be a flavoprotein (absorption bands at 460 and 280 mμ) and contains catalase (Kat. f. 57). Its pH optimum is between 4 and 6. Despite impurity, it has yielded accurate and theoretical results. Recoveries of glucose added to blood and to incubation media (containing other sugars and glucose phosphates) ranged from 96.0%–101.0%. The technic is particularly useful in 3 situations: *a*) De-

termination of residual glucose in Warburg flasks following tissue incubations. Glucose oxidase (usually 0.3 ml. 0.1% buffered solution) is introduced into the sidearm, tipped and incubated 2 hours. Data show that true glucose disappearance may differ significantly from reducing activity disappearance especially with tissues of low metabolic activity such as leukocytes. *b*) Determination of hydrolytic enzymes liberating glucose such as glucose-6-phosphatase. Data show accuracy of method and advantages of continuous curves permitting study of kinetics. *c*) Blood sugar determination. Comparison of available methods indicates glucose oxidase gives lowest values, 0.1–3.0% lower than $Cd(OH)_2$-ferricyanide method of Kingsley and Reinhold. (Based on work performed under Contract No. AT-04-1-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.)

Chromatographic separation studies on products of ribonuclease action. RONALD A. BECKER* AND FRANK WORTHINGTON ALLEN. *Div. of Biochemistry, Univ. of California Med. School, Berkeley.*

The substance produced by the action of ribonuclease upon a sample of purified, dialyzed ribonucleic acid from yeast have been separated into a dialyzable fraction, and a non-dialyzable residue. The dialyzable material has been partially analyzed by a two dimension paper chromatographic technique. The isobutyric acid-ammonia solvent of Magasanik *et al.* (*J. Biol. Chem.* 186: 37, 1950) was used in the first dimension, while the tertiary amyl alcohol-water-formic acid solvent of Hanes and Isherwood (*Nature* 164: 1107, 1949) was used in the second dimension. Absorbing material was located by ultraviolet light photography, and the compact spots eluted with a 0.05 M phosphate buffer of pH 7.0 for 3 hours at 40°. Mononucleotides were identified by chromatographic comparison with the nucleotides as isolated classically by Levene, and verified by ultraviolet spectroscopy. The following proportions of the nucleotides, given as percentages of the dry weight of the total digest were present: cytidylic acid, 6; uridylic acid, 5.9; guanylic acid, 0.6; and adenylic acid, 0.4. Nucleosides were present in the digest in an amount less than 1%. Four uncharacterized spots were present on the chromatograms. The chromatographic behavior and dialysis rate of the substances comprising these spots indicate that they are polynucleotides of low molecular weight. Thus, the total products of ribonuclease action on this sample of ribonucleic acid are 13% mononucleotides, 39% small polynucleotides and 47% larger polynucleotides.

Evidence for existence of an apoerythein precursor in gastric mucosa. ERNEST BEERSTECHER, JR. AND ELEANOR J. EDMONDS.* *Dept. of Biochemistry, Univ. of Texas School of Dentistry, Houston.*

Apoerythein, a protein which combines firmly and stoichiometrically with vitamin B_{12}, exists in the saliva, in the gastric juice, and in gastric and duodenal mucosa. It is closely related to, if not identical with, Castle's intrinsic factor. The thermal stability of salivary apoerythein differs from that of mucosal apoerythein because of the presence of a heat sensitized apoerythein inactivator in saliva. The thermal stability of gastric juice apoerythein varies with the pH at which it is heated, but agrees well with published values obtained by clinical tests on the intrinsic factor. Gastric mucosal apoerythein is acid labile, thereby differing from gastric juice apoerythein. Achlorhydric gastric juice contains an acid-labile apoerythein inactivating enzyme which accounts for the absence of apoerythein from the gastric juice of patients with pernicious anemia and some other gastric disorders. Both chemical and clinical results suggest strongly the identity of apoerythein with intrinsic factor, and data are cited to account for evidence which would seem to indicate otherwise. The relationship of apoerythein to vitamin B_{12} metabolism and the etiology of pernicious anemia is discussed.

Metabolism of niacin induced growth. WILLIAM T. BEHER* AND O. H. GAEBLER. *Edsel B. Ford Inst. for Med. Research, Henry Ford Hosp., Detroit, Mich.*

In dogs, excretion of N^1methylnicotinamide (NMeN) is greatly diminished during nitrogen storage induced by testosterone propionate (TP) or growth hormone (*J. Nutrition* 41: 447, 1950). The effect of TP, injected intramuscularly, on NMeN excretion and on the concentration of coenzymes I and II in liver and kidney has been studied further in female Sprague-Dawley rats, 4 months old. Food intake was kept constant and identical in all animals whether treated or untreated. When 0.5 mg. TP was administered daily, the output of NMeN fell promptly and remained low for as long as 12 days before returning to the control level. When the daily dose of TP was increased to 6.25 mg., NMeN excretion decreased at first, then rose to twice the control value. The average concentration and the total amount of coenzymes I and II in the organs was determined in groups of 10 to 14 rats which received 0, 0.5, or 6.25 mg. of TP daily for 10 days. In the kidney the average concentration was unaltered, while the total amount increased 17% and 39% respectively in the treated groups, due to the effect of TP on organ weight. In the liver, TP increased the concentration of the coenzymes an average of 14%, and the total amount 43% and 64% respectively,

in the two treated groups. These findings indicate that the decreased excretion of NMeN in the treated animals is due in part to increased synthesis of coenzymes.

Convenient method for preparation of coenzyme A. H. Beinert,* R. Van Korff,* D. E. Green, D. A. Buyske,* R. E. Handschumacher,* H. Higgins* and F. M. Strong. *Inst. for Enzyme Research and Dept. of Biochemistry, Univ. of Wisconsin, Madison.*

A method will be described which leads in one purification step from crude concentrates of coenzyme A (CoA) to preparations of high purity, which should be practicable in any laboratory. Concentrates are obtained by charcoal absorption of CoA from extracts (100°) of dried brewer's yeast, pyridine elution and acetone precipitation from the concentrated eluate. The final purification step consists in a specific co-precipitation of CoA with carrier glutathione (GSH) in strong acid solution by Cu_2O and achieves some 30-fold increase in purity. The concentrate of GSH and CoA obtained after washing and decomposing the precipitate with H_2S is suitable for most enzymatic studies requiring CoA, although GSH may be easily removed with Dowex 50. Preparations containing from 12–24% bound pantothenic acid (by transacetylase, sulfanilamide, and microbiological assays) were obtained in 10–15% overall yield, depending on the purity of the original concentrate. No contaminant of nucleotide character but some residual glutathione (ca 5%) has been found. Now that purified CoA has been made available in quantity, the way is open for the preparation of CoA derivatives of biological importance.

Mechanism of inactivation of mercurial diuretics by BAL. Reinhold Benesch and Ruth E. Benesch (introduced by Irving M. Klotz). *Dept. of Biochemistry, Northwestern Univ. Med. School, Chicago, Ill.*

It has been demonstrated in several laboratories that the diuretic effect of mercurated allylamides is effectively suppressed by BAL. The chemical reaction between these compounds and BAL was therefore subjected to a detailed study using recently developed polarographic methods, which permit the direct assay of organically bound mercury. Further work has shown that it is also possible to determine both the free mercurials and the mercaptides resulting from their interaction with —SH groups in the presence of each other. The reaction between a typical diuretic, Salyrgan, and BAL was found to proceed in 2 stages, the first one leading to the formation of an unstable soluble dimercaptide, which is, however, sufficiently stable at 0°C. to permit its polarographic characterization. In the second stage the dimercaptide decomposes to form *1*) 1 mole of an insoluble, cyclic mercuric mercaptide of BAL, *2*) 1 mole of free Salyrgan, *3*) 1 mole of salicylallylamido-o-acetic acid, and *4*) 1 mole of methanol. This mechanism was deduced by polarographic determination of the concentrations of free Salyrgan and Salyrgan dimercaptide during the course of the reaction. In addition, some of the other reaction products, i.e. the cyclic BAL mercaptide and salicylallylamido-o-acetic acid were isolated and characterized by analysis. The rate of the reaction was found to vary greatly with pH. It was followed as a function of pH at 26°C. and 37.5°C. both by the diminution in the height of the dimercaptide wave and the increase in the height of the wave due to free Salyrgan. The reaction was found to be first order under all conditions studied. The plot of the log of the first order rate constants against pH revealed that 1 hydrogen ion reacts with 1 mole of dimercaptide to give the reaction products listed above. The destruction of the organic mercury molecule by BAL and its conversion into an insoluble cyclic BAL complex thus provides a rational chemical explanation for the suppression of mercurial diuresis by this dithiol. (Supported by a research grant from the National Institute of Health, U. S. Public Health Service.)

Synthesis of methionine in cell free extracts. Paul Berg (introduced by Charles E. Carter). *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

Previous studies (*J. Biol. Chem.* 190: 31, 1951) have indicated the existence of a pathway for the synthesis of methionine, which is independent of the formation of choline or betaine methyl groups. Further studies of this reaction have been carried out using cell free extracts of pigeon liver. These preparations are capable of converting C^{14} formate and formaldehyde to the methyl group of methionine in the presence of homocysteine. The extracts prepared by high speed centrifugation of pigeon liver homogenates are stable in the lyophylized state but rapidly lose activity after brief periods of dialysis at 2°C. The activity can be partially restored by addition of boiled extracts of fresh pigeon liver. Chromatographic separation of the products formed after incubation with C^{14} formate has demonstrated the accumulation of an unidentified component which accounted for a major portion of the formate which disappeared. This compound does not accumulate when homocysteine is omitted from the incubation. Cysteine, glutathione and ascorbic acid do not substitute for homocysteine in promoting the formation of this compound. The radioactive compound on the chromatogram gives a positive

ninhydrin color. This association remains constant both after chromatographing in several solvents, and after eluting the compound and rechromatographing the eluate. The nature of this compound and its relation to methionine synthesis is being investigated.

Influence of fluorides on mechanism of tooth development. M. K. BERKUT* AND JAMES C. ANDREWS. *Dept. of Biological Chemistry and Nutrition, Univ. of North Carolina School of Medicine, Chapel Hill.*

The effect of different levels of fluoride intake on the deposition of potassium in tooth tissue has been studied indirectly by the substitution of radioactive rubidium (Rb^{86}) for radioactive potassium (K^{42}). This substitution was made because of the much longer half-life of Rb^{86} (19.5 days) as compared with that of K^{42} (12.4 hours). Administration of sodium fluoride to growing hamsters 24 hours after a single injection of Rb^{86} caused the excretion of only 4–6% of the injected rubidium; control animals (no fluoride) excreted in the same period, 16–23% of the injected rubidium. Analyses of various body tissues after such experiments substantiated the conclusion as to the much greater retention of Rb^{86} after fluoride administration. Total recovery of all radioactivity ranged from 99–104%. The effect on the potassium, calcium and phosphorus content of the hamsters' teeth caused by levels of fluoride in drinking water varying from 5–50 p.p.m. F was investigated. At 50 p.p.m. F there was no significant difference between experimental animals and controls (no fluorine) as regards calcium and phosphorus. However, the high fluorine intake raised the potassium content of the lower central incisors by from 66–123% over that of the controls. Other teeth showed similar effects. (Work performed under Contract AT-(40-1)-299, Title II, with the U. S. Atomic Energy Commission.)

β-Glucuronidase of calf liver. PETER BERNFELD,* JEROME S. NISSELBAUM* AND WILLIAM H. FISHMAN. *Cancer Research and Cancer Control Unit, Tufts College Med. School, Boston, Mass.*

β-Glucuronidase, extracted from fresh calf liver, has been purified according to the method outlined for the purification of calf spleen β-glucuronidase (*Arch. Biochem.* 27: 475, 1950). In order to adapt the method for the liver enzyme, slightly modified fractionation limits were employed. After lyophilization of the product, a white powder is obtained which is readily soluble in dilute buffer solutions. 1 γ of this enzyme hydrolyzes 12.5 γ phenolphthalein glucuronide in a total volume of 1 ml. in 10 minutes at 37°C. at pH 4.5, when desoxyribonucleic acid (DNA) has been added. The enzyme preparation is very stable between pH 3.1 and pH 7.0. It has a maximum of light absorption at 278 mμ and, on electrophoresis, it migrates as a single component at pH 3.5. The enzymatic activity of this preparation is markedly increased by the addition of commercial DNA. This phenomenon is particularly striking in very dilute enzyme solutions. From kinetic experiments it can be concluded that calf liver glucuronidase dissociates on dilution into an inactive mixture of apo- and coenzyme, and that the addition of DNA to this system has the same effect as if the coenzyme concentration were increased, thus yielding more holoenzyme. The effects of dilution, and of activation by DNA found with calf liver β-glucuronidase, markedly resemble those previously reported in experiments with purified calf spleen β-glucuronidase (*Science* 112: 653, 1950).

***In vivo* synthesis of ribose studied with radioactive carbon.** I. A. BERNSTEIN (introduced by J. A. MUNTZ). *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

The *in vivo* synthesis of ribose has been studied in the chick by feeding C^{14}-labeled compounds and comparing the tracer distribution in the glycogen and ribose to determine if hexose is a direct precursor of the pentose via removal of an end carbon. When acetate-1-C^{14} was fed, the tracer patterns in the ribose of the nucleic acid purine nucleotide fraction and in the glycogen were found to be markedly different (BERNSTEIN. *J. Amer. Chem. Soc.* 73: 5003, 1951). It was concluded from the results that under these conditions hexose was not a major direct precursor of the pentose. The data obtained were not inconsistent, however, with a C_3 and C_2 condensation mechanism. This work has now been extended by feeding formate-C^{14} under similar conditions and the previous results have been confirmed in that different tracer patterns were found in the glycogen and ribose. It has further been noted in preliminary studies that the feeding of non-labeled glycine increases the incorporation of C^{14} from formate into the pentose. The tracer distribution patterns in the hexose and pentose after feeding formate-C^{14} plus non-labeled glycine as well as after feeding glycine-2-C^{14} alone are being investigated.

Glutamate stimulation of malate and fumarate oxidation in brain homogenates. SAMUEL P. BESSMAN (introduced by SIDNEY UDENFRIEND). *Research Fndn. of Children's Hosp., Washington, D. C.*

KCl homogenates of rat or mouse brain, dialyzed against phosphate buffer and fortified with ATP and DPN, do not oxidize malate and fumarate readily. The addition of 0.01M glutamate increases the oxidation tenfold or more. In the presence of arsenite ketoglutarate accumulates, proportional to this increased oxidation. This effect

can be shown to be caused by a transamination between glutamate and oxalacetate, since fumarate disappearance can be accounted for completely by malate, aspartate, and oxalacetate (as pyruvate) formation, and glutamate can be accounted for as ketoglutarate. The same effect can be demonstrated with extracts of dialyzed brain by following the reduction of DPN spectrophotometrically in the presence of cyanide. Fumarate or malate alone cause little reduction, but addition of glutamate causes rapid reduction. Conversely, glutamate alone causes little reduction, but addition of fumarate or malate causes the same increase in rate. Since the concentration of glutamate in brain is 0.01 M these results suggest that glutamate might exert a significant effect on the Krebs cycle oxidations in brain. No citrate formation could be demonstrated in these experiments, and the difficulty in demonstrating citrate formation in brain under any circumstances might be caused in part by this shunt.

Diurnal trend and variation of urinary creatinine excretion. WILLIAM R. BEST,* W. JAMES KUHL, JR.* AND THEODORE E. FRIEDEMANN. *U. S. Army Med. Nutrition Lab., Chicago, Ill.*

Five 27–32 year old normal white males of average build, while following their usual diet and activities, collected 6 4-hour urine specimens daily over a period of 14–20 days. The mean 24-hour creatinine excretion varied from 1.64–2.01 gm. The coefficient of variation for repeated 24-hour collections was from 5.8%–12.1%, with a mean of 9.1%. The range of individual 24-hour excretions was from 23.0% above to 20.4% below the mean rates. The mean fractional excretions described a significant diurnal cycle in each subject, although individual values were often divergent. The lowest mean excretion of 13.5% less than the 24-hour rate occurred from 2–6 A.M. The highest rates fell between 2 and 10 P.M. with a mean peak of plus 6.8% at 2–6 P.M. The 6–10 A.M. specimen reflected the 24-hour rate more closely than did the other fractional or the eight-hour overnight specimen. This period also showed the least inter-individual variation, mean values ranging from 1.6% above to 3.0% below the respective 24-hour rates. The coefficient of variation for repeated collections during this interval was generally lower than for other periods, with a range of 5.9%–15.0% and a value of 11.6% for the pooled data of all subjects. The average of 2 successive 6–10 A.M. collections gave individual coefficients of variation of 5.7%–11.7% with pooled value of 8.9%. Similar data using creatinine-free diets will be presented.

Activators of hydrolysis of glutathione. FRANCIS BINKLEY AND G. M. CHRISTENSEN.* *Emory Univ. School of Dentistry, Emory University, Ga.*

Previous reports have described the activation of the enzymatic hydrolysis of glutathione by glutamine; other amino acids and amides of amino acids appeared to be without effect. In view of the results of Hanes, Hird and Isherwood (*Nature* 166: 288, 1950) such specificity would not be expected. Further studies have revealed that certain L-amino acids (particularly methionine) are activators whereas D-amino acids are inhibitory and DL-mixtures are essentially without effect. Glycine was without effect in the absence of a preparation of dipeptidase activities (cysteinylglycinase) but was found to activate in the presence of this second enzyme. Glycylglycine was the most active compound tested whereas glycineamide, glycine ethyl ester and glycylglycylglycine were without effect. Other dipeptides were prepared and although some were active beyond the effects of the component amino acids, the effects were of much smaller magnitude than those of glycylglycine. The reaction mixtures with glycine and glycine peptides were investigated by paper chromatography for the presence of γ-glutamyl peptides and presumptive evidence for the presence of γ-glutamyl dipeptides and tripeptides of glycine was obtained. It would appear that the hydrolysis of glutathione by the enzyme of kidney tissue is a transpeptidation reaction and that the activation of the hydrolysis by preparations of dipeptidases (cysteinylglycinase) may be explained as the result of the synthesis of active dipeptides from the constituent amino acids of glutathione.

Solubility of estrone in biologic systems. FRITZ BISCHOFF AND ROBERT E. KATHERMAN.* *Santa Barbara Cottage Hosp. Research Inst., Santa Barbara, Calif.*

Solubility determinations at 37.5°C. of estrone, m.p. 258.5°C., in isotonic saline, in 3.1% crystalline bovine albumin solution(μ = 0.155), and in rabbit and human sera were made by procedures previously reported (*Am. J. Physiol.* 152: 189, 1948; 164: 774, 1951). Equilibrium was attained in saline in less than 90 hours and in the protein solutions in less than 30 hours. The solubility in saline was $(5 \pm 0.5) \times 10^{-6}$ moles/l.; a shift of pH from 6.5–8.1 was without influence. The dispersion in albumin solution, pH 5.3, was $(5.9 \pm 1.0) \times 10^{-5}$ moles/l. and at pH 8.5, and pH 8.8, $(1.6 \pm 0.2) \times 10^{-4}$ moles/l. Solubilities in human serum, 7.2% protein, and in rabbit serum, 7.9% protein, were respectively $(1.37 \pm 0.2) \times 10^{-4}$ and $(1.14 \pm 0.2) \times 10^{-4}$ moles/l. In similar experiments for estradiol, the solubilities in human serum, 6.9% protein, and rabbit serum, 6.9% protein, were respectively $(3.6 \pm 0.2) \times 10^{-4}$ and $(4.3 \pm 0.3) \times 10^{-4}$ moles/l. The solubility of estradiol in 3.1% albumin solution, pH 8.5, was

4.9×10^{-4} moles/l. Solubility of estrone palmitate, m.p. 73.5–74.0°C., was determined in 3.2% bovine albumin, pH 7.5, and in a mixture of bovine plasma proteins and lecithin, pH 7.4, (4.0% albumin, 1.0% fraction III-I, 1.0% γ globulin and 0.25% lecithin, both solutions at $\mu = 0.155$). The solubilities were in all cases less than the sensitivity of the method, 0.7×10^{-5} moles/l. (Supported in part by a grant C 1176 C from the National Cancer Institute, of the National Institutes of Health, U. S. Public Health Service. Acknowledgments to Parke, Davis and Company for estrone, Armour and Company for proteins, and Schering Corporation for estradiol.)

Solubilities of testosterone and estradiol esters in biologic systems. FRITZ BISCHOFF, ROBERT E. KATHERMAN,* YEE SING YEE AND JOHN J. MORAN.* *Santa Barbara Cottage Hosp. Research Inst., Santa Barbara, Calif.*

The solubilities at 37.5°C. of testosterone propionate, m.p. 119.6–120°C., and of testosterone palmitate, m.p. 73.5°C., were determined by methods previously described for testosterone (*J. Biol. Chem.* 174: 663, 1948). In water from undersaturation, the solubility of testosterone propionate, $(1.06 \pm .08) \times 10^{-5}$ moles/l., at 233 hours equilibration equalled the value approached from supersaturation (4 hours at 80°C., then 72 hours at 37.5°C.). Equilibrium was reached for testosterone propionate in 2.9% albumin solution ($\mu = 155$) in 48 hours; the values for pH 8.3 and pH 5.3 were respectively 6.2×10^{-4} and 4.0×10^{-4} moles/l. Equilibrium was not reached in 72 hours in either 1.9% γ globulin or 2.1% III-I lipoprotein. Limiting values of 2.2×10^{-4} for γ globulin and 3.3×10^{-4} for III-I protein were obtained in the pH range 7.1–7.4. The solubility of testosterone palmitate in 3.2% bovine albumin, pH 7.7, and for a mixture of proteins and lecithin approaching the composition of serum was $(2.1 \pm 0.5) \times 10^{-5}$. Solubility determinations of testosterone in the same solvents were also made. The results at 24 hours equilibration were in excellent agreement with the 48 hour values and follow as moles/l.: in 0.25% lecithin, pH 7.0, 2.6×10^{-4}, in 1.9% III-I lipoprotein, pH 7.4, 3.8×10^{-4}, and in the lecithin protein mixture, pH 7.5, 18.6×10^{-4}. The solubilities at 37.5°C. of estradiol benzoate, m.p. 191.1–193.2°C., were determined by bioassay. The results in those experiments in which equilibrium was reached follow in moles/l.: 2.9% albumin, pH 5.3, $(6.1 \pm 0.8) \times 10^{-6}$, 0.2% lecithin, pH 6.8, 5.5×10^{-6}. (Supported in part by a grant C 1176 C from the National Cancer Institute, of the National Institutes of Health, U. S. Public Health Service. Acknowledgment is made to Schering Corporation for steroids.)

Mechanism of ATP-acetate reaction. SIMON BLACK, MARY ELLEN JONES,* RUTH M. FLYNN* AND FRITZ LIPMANN. *Biochemical Reserach Lab., Massachusetts General Hosp., and Dept. of Biological Chemistry, Harvard Med. School, Boston.*

Enzyme preparations from pigeon liver and from yeast were used. The yeast enzyme is better suited for purification. The rate of acetahydroxmic acid formation was used as a measure of enzyme activity with 0.2 M hydroxylamine and catalytic amounts (25 units/ml.) of coenzyme A (CoA) present in the enzyme system. The reaction is dependent on the presence of magnesium ion. A 25-fold purification of the yeast enzyme is obtained with nucleic acid and protamine fractionations. If substantial amounts of CoA are used with such a concentrated enzyme in the absence of hydroxylamine, an almost quantitative conversion to acetyl-CoA is observed. The acetyl-CoA is measured by the hydroxamic acid method after termination of the experiment. With active enzyme preparations, the acetylation of CoA in amounts of 2–4 μM/ml. occurs very rapidly and may be completed in 5 minutes or less. A further purification of the enzyme system and a study on the mechanism of the reaction are in progress.

Effect of depletion of amino acids on phage production in a lysogenic organism. ERNEST BOREK. *Service of Microbial Physiology, Pasteur Inst., Paris.*

The lysogenic condition of certain microorganisms—their ability to produce bacteriophage without the intervention of external phage—has been unequivocally established, and the field has been opened to quantitative biochemical studies by Lwoff's discovery that phage formation can be induced by subjecting such organisms to environmental stresses (ultraviolet irradiation, sulfhydryl compounds). (LWOFF, A. *et al. Ann. Inst. Pasteur* 78: 1, 1950; 79: 815, 1950). The effect of starvation of amino acids on the ability to respond to ultra violet irradiation by phage formation was studied in lysogenic *E. coli* K-12 which also have amino acid synthesizing deficiencies. Depletion of essential amino acids prior to irradiation suppressed the ability of the organism to respond to u. v. irradiation by phage production. Doses of u. v. which normally precipitate phage production were without measurable effect on organisms starved of essential amino acids for three hours prior to the irradiation. Such resistance to irradiation was not the result merely of diminished metabolism, for organisms kept at 0°C. for as long as 24 hours responded to irradiation by phage production. Starvation of the organisms after irradiation had no effect on their response to irradiation. If the lacking amino acid was restored as late as 3 hours after the irradiation, phage production ensued

as usual. (The work was carried out during the tenure of a Fellowship from the John Simon Guggenheim Memorial Foundation.)

Nitrogen balance and lysine deficiency. JAMES W. BOTHWELL* AND J. N. WILLIAMS, JR. *Dept. of Biochemistry, Univ. of Wisconsin College of Agriculture, Madison.*

Nitrogen excretion studies were made with weanling male rats in comparing the effects of a lysine deficiency when the ration was fed ad libitum or force-fed by stomach tube. Suitable controls receiving a complete ration were included in each group. Animals receiving the deficient ration ad lib. soon exhibited the expected loss of appetite and weight with a negative nitrogen balance which continued throughout the 14-day experimental period. As previously noted in similar experiments with a methionine or histidine deficiency, the results with a lysine deficiency showed a marked correlation between nitrogen intake and nitrogen balance. Animals force-fed the lysine deficient ration remained in positive nitrogen balance even until time of death which usually occurred after 9–14 days. In contrast, however, to the rather constant urinary and fecal excretion of nitrogen found in the methionine and histidine studies, a marked increase of urinary nitrogen occurred after the first few days in the group force-fed the lysine deficient ration followed by a decrease in urinary nitrogen as the animals exhibited marked deficiency symptoms. An increase in fecal nitrogen was also observed as the experiment progressed. Therefore, the nitrogen balance of the force-fed lysine deficient group first decreased toward negativity but later became strongly positive as the deficiency was continued.

Biosynthesis of radioactive long chain fatty acids by homogenized pigeon liver. ROSCOE O. BRADY* AND SAMUEL GURIN. *Dept. of Physiological Chemistry, Univ. of Pennsylvania School of Medicine, Philadelphia.*

Further studies have been carried out utilizing the previously described cell-free preparation of homogenized pigeon liver tissue which is capable of incorporating C^{14}-labeled acetate into long chain fatty acids (*Arch. Biochem. and Biophys.* 34: 221, 1951). The recovered fatty acids contain a slight preponderance of C^{14} in the carboxyl or alpha carbon. This suggests that while these fatty acids are predominantly synthesized from 2-carbon fragments, there is a small amount of coupling to non-labeled long chain fatty acids. It has been found that the mitochondrial fraction combined with the supernatant provide the essential components of the system. Significant stimulation is obtained by the addition of Mg ions or oxalacetate. Maximal effects are obtained when both are employed. An inhibition is observed with Ca ions, citrate, fluoride, and to some extent with ATP. Dinitrophenol, insulin, and growth hormone exert little or no effect when added to the system. The supernatant fraction cannot be replaced by a water soluble extract of homogenized pigeon breast muscle.

Effects of l-glutamine and methionine sulphoxide on inhibition of acetylcholine synthesis in brain by ammonium ions. B. M. BRAGANCA AND P. FAULKNER (introduced by J. H. QUASTEL). *Research Inst., Montreal General Hosp., Montreal, Canada.*

It is known (MANN, TENNENBAUM AND QUASTEL, *Biochem. J.* 33: 822, 1939), that ammonium ions exert an inhibitory effect on aerobic acetylcholine synthesis, in the presence of glucose, in brain *in vitro*. It is possible that the ammonia inhibition is due to its conversion of α-ketoglutaric acid, produced during the oxidation of glucose in brain, into glutamate and thence into glutamine, thereby reducing the level, in the cell, of ATP, and therefore, diminishing the rate of acetylcholine synthesis. If this view is correct, it might be expected that, in the presence of excess glutamine, the synthesis of the latter would be decreased and ATP formation would not be reduced to the same extent. Experiments with rat brain cortex slices show that in presence of 0.027M ammonium ions, acetylcholine synthesis is depressed by 70%, but that if l-glutamine (.01M) is added at the same time, the depression of synthesis is 40%. Glutamine, in effect, stimulates acetylcholine formation in presence of ammonium ions and glucose by at least 85%. Further experiments indicate that methionine sulphoxide which specifically inhibits the formation of glutamine from glutamate and ammonium ions (WAELSCH *et al. J. Biol. Chem.* 166: 273, 1946) also stimulates the synthesis of acetylcholine in presence of ammonium ions. These results are of interest in the study of acetylcholine synthesis in brain during convulsions, since under these conditions ammonium ions are known to accumulate in the brain.

Effects of x-irradiation on activity of tissue transaminases. MYRON BRIN (introduced by RALPH W. MCKEE). *Cancer Research Inst., New England Deaconess Hosp., Boston, Mass.*

The activity of glutamic-aspartic and glutamic-alanine transaminases has been studied in excised tissues of untreated and total body x-irradiated (1500r) male albino rats, at various intervals after exposure. Homogenates of heart, liver, duodenum and spleen were assayed as described previously (BRIN, M. AND R. E. OLSON. *Federation Proc.* 10: 166, 1951). A transaminase was computed on the basis of tissue wet weight. Of the 4 tissues studied only heart was resistant to change in transaminase

activity. Evidences of metabolic alteration by x-rays were most prominent in duodenum in which the glutamic-alanine activity dropped to 5% of normal at 4–6 days after exposure and reverted to only 30–40% of normal in 10 days. Of particular interest was the effect on liver in which it was observed at 2–5 days after exposure that both transaminases were stimulated to levels 30–50% higher than the controls. This may be related to the increased glycogenesis observed in x-irradiated rats. After 7 days, however, the glutamic-alanine enzyme activity in liver dropped below normal. Although transamination in spleen of both control and irradiated animals were variable, the data indicate depressions in the activity of both enzymes. It is significant that the glutamic-alanine system was affected more markedly than the aspartic enzymes. Humoral effects on transamination in tissues is being investigated by the partial shielding of single rats and parabionts.

Bacterial DPN-cytochrome c reductase. ARNOLD F. BRODIE AND JOSEPH S. GOTS (introduced by M. G. SEVAG). *Dept. of Microbiology, Univ. of Pennsylvania School of Medicine, Philadelphia.*

DPN-cytochrome c reductase in animal tissue and yeast have previously been described. We have obtained an active enzyme preparation with a similar function from a sonically vibrated suspension of *Escherichia coli* (strain ECFS). The activity was determined spectrophotometrically by measuring the increase in absorption of reduced cytochrome c at 550 mμ and decrease of reduced DPN at 340 mμ, in presence of chemically reduced DPN and the bacterial reductase. In absence of cytochrome c, reduced DPN is slowly oxidized in presence of oxygen or methylene blue. It was likewise found that neotetrazolium, 2,3,5-triphenyltetrazolium chloride, and 5-nitro-2-furaldehyde semicarbazone (Furacin) functioned as electron acceptors. The reduction of neotetrazolium, which was studied more extensively, was found to be dependent on the presence of added FAD to an inactive apoenzyme prepared by dialysis in HCl-KH_2PO_4 buffer. Further evidence for the flavoprotein nature of this enzyme was provided by the ability of heated reductase to activate D-amino acid oxidase apoenzyme in manometric systems. In absence of reduced DPN, alcohol, acetaldehyde, lactate, succinate, malate, fumarate or pyruvate failed to serve as substrates for this enzyme. While 0.001 M concentrations of cyanide, azide, urethane and optochin failed to inhibit, 0.001 M of atabrine and acriflavin caused 70 and 15% inhibition, respectively. Data will be presented on the kinetics of this enzyme and its effects on reduced TPN.

Involvement of citrovorum factor in synthesis of histidine in yeast. HARRY P. BROQUIST. *Lederle Labs. Div., American Cyanamid Co., Pearl River, N. Y.*

Sprinson (Abstracts, Am. Chem. Soc., 119th meeting, April 1951) has shown that histidine 2-C^{14} furnishes 'formate' for the ureide carbon atoms of uric acid, and Levy and Coon (*J. Biol. Chem.* 192: 807, 1951) reported that when yeast is grown with formic acid-C^{14}, the formate is incorporated specifically into the amidine carbon of the imidazole ring histidine. It seemed possible that folic acid might function in the synthesis of histidine in view of the evidence linking folic acid to reactions involving a 'single-carbon fragment.' When the yeast *Torula cremoris* (ATCC 2512) was grown on a simple synthetic medium, it was found that 4-aminopteroylglutamic acid (Aminopterin) was extremely inhibitory for growth of the organism, but this toxicity could be reversed in a competitive fashion by concentrates of the citrovorum factor (CF) a biologically functional form of folic acid. It was also found that the toxicity of Aminopterin for growth of *T. cremoris* could be reversed in a non-competitive fashion by the stepwise addition of methionine; methionine + purine bases; and methionine + purine bases + histidine; with each addition the toxicity of Aminopterin was successively diminished. These findings are interpreted to mean that Aminopterin interferes with reactions in the cell catalyzed by CF which are essential for growth; thus the inhibition may be overcome by adding sufficient CF, or by adding the products of the action of CF. The data imply that in growing cells of *T. cremoris* CF is concerned in the synthesis of methionine, purine bases, and histidine.

Free amino groups of human adult hemoglobin. HENRY BROWN (introduced by JOHN G. REINHOLD). *Dept. of Chemistry, Gannon College, Erie, Pa.*

During the course of studies of blood proteins, we have had occasion to re-investigate the free amino groups of 'normal' human adult hemoglobin using Sanger's DNP-technique. Our first specimens of hemoglobin from 3 different people yielded approximately 2 terminal valine residues/molecule (2.3, 2.0, 2.1) and about 43 lysines (46, 41, 42). On further investigation it was found that by using other, and perhaps more potent preparations of dinitrofluorobenzene, the reaction could be forced to proceed further. About 5 terminal valines could then be identified, and the same lysine content as previously, taking into account the errors of the method. For example, using a specimen from the first of the above patients we now found 4.8 valines and 40 lysines. The findings were confirmed with other samples of human adult hemoglobins. Likewise, with horse hemoglobin we found first 3.2 valines, 40 lysines; then with the same

sample, causing the reaction to proceed, 5.9 valines and 44 lysines. These higher valine values agreed with the original reports of Porter and Sanger (*Biochem. J.* 42: 287, 1948) who first used this technique with hemoglobins. The data indicate that in the specimens studied one may obtain either 2 or 5 terminal moles of valine/mole of human adult hemoglobin. This difference may well only be a matter of completeness of reaction. (We gratefully acknowledge the support given this work by a contract with the Office of Naval Research, and by a grant from the American Cancer Society.)

Effect of some dietary organic peroxides on *N*-demethylation of 3-methyl-4-monomethylaminoazobenzene. R. R. Brown,* J. A. Miller and E. C. Miller. *McArdle Memorial Lab., Univ. of Wisconsin, Madison.*

Earlier studies showed the presence of a dietary factor in certain crude products which increased by approximately 250% the ability of mouse liver homogenates to N-demethylate 3-methyl-4-monomethylaminoazobenzene. (Miller, Brown, Mueller and Miller. *Cancer Res.* 11: 269, 1951). Similar increases in demethylation activity can be obtained by the addition of low levels of certain organic peroxides or compounds capable of forming peroxides to the diet. These compounds include aged crude cholesterol or hydrogen peroxide-treated samples of a number of purified sterols; the purified sterols were inactive prior to treatment. Ascaridole and 9,10-dimethyl-1,2-benzanthracene-photoöxide, both relatively stable peroxides, were active at dietary levels of 0.01–0.10%. 9,10-Dimethyl-1,2-benzanthracene was inactive at levels of 0.01–0.054%, while 20-methylcholanthrene, pyrene, and 1,2-benzanthracene were active at levels of 0.05–0.10%. The assays were conducted as described previously on the livers of weanling mice prefed for 1 week on a purified diet and then fed the same diet plus the test compound for an additional week. These findings are of added interest in view of the observation of Richardson and Cunningham (*Cancer Res.* 11: 274, 1951) that rats treated simultaneously with the hepatic carcinogen 3′-methyl-4-dimethyl-aminoazobenzene and 20-methylcholanthrene develop liver tumors much more slowly than those fed the dye alone. Other hydrocarbons also inhibit tumor induction by 3′-methyl-4-dimethylaminoazobenzene and the hydrocarbons appear to act by helping to maintain the ability of the liver to N-demethylate and reduce the azo dyes (Miller, Miller and Brown. *Cancer Res.* in press). Hydrocarbon peroxides are likely intermediates in this process.

Identification of n-valeric acid as a metabolic product of bacteria-free *Ascaris lumbricoides*. Ernest Bueding. *Dept. of Pharmacology, Western Reserve Univ., Cleveland, Ohio.*

The production of racemic α-methylbutyric acid by bacteria-free *Ascaris lumbricoides* has been reported recently (E. Bueding and H. W. Yale: *J. Biol. Chem.* 193: 411, 1951). Media in which bacteria-free *Ascaris* had been incubated contain 2 other volatile acids whose solubility characteristics are similar to those of α-methylbutyric acid. Separation of these acids by partition chromatography will be described. One of them has the properties of an α-β-unsaturated acid; after esterification with p-bromphenacylbromide, the other has been identified as n-valeric acid. Formation of the latter by *Ascaris* is about 50% lower than that of α-methylbutyric acid. (This investigation has been supported by a grant from the U. S. Public Health Service.)

The non-protein moiety of phosphorylase. Mary V. Buell. *Dept. of Pharmacology, Washington Univ. School of Medicine, St. Louis, Mo.*

The non-protein fraction of 400 mg. (1 micromole) of 7 times crystallized phosphorylase, prepared from rabbit muscle, was chromatographed on Dowex 1. Several points of interest were found: *1*) Uridylic acid was isolated from the eluate by a new reaction, and identified. *2*) A yellow pigment was isolated and tentatively identified as 2 methyl, 1, 4 Naphthoquionone. *3*) Cystine was isolated in sufficient quantities to suggest that cysteine may occur as an end group in the protein molecule and serve as a connecting link with the prosthetic group. (Supported in part by a grant from the American Cancer Society.)

Microfluorometric measurement of phosphopyridine nucleotides in blood serum and cells. Helen B. Burch (introduced by C. G. King). *Dept. of Chemistry, Columbia Univ., New York City.*

By the condensation of phosphopyridine nucleotides and N-methyl nicotinamide with methyl ethyl ketone in alkaline solution, it was possible to measure by fluorescence the amounts of these compounds in filtrates corresponding to 2 cu. mm. of whole blood, 25 cu. mm. of serum, or in cells obtainable from 0.1 ml. of blood. By means of this reaction, the decrease of pyridine nucleotides, calculated as DPN, was followed in the blood of rats receiving a corn grits diet. The blood levels of those receiving the same diet plus supplements of equivalent amounts of nicotinic acid, nicotinamide, and 3-pyridine methanol tartrate were also studied. All of these supplements were effective in maintaining satisfactory levels of PN in rat blood. After 6 weeks, blood levels for all the groups receiving the supplemented diets were almost twice that of groups receiving the corn grits diet. Average weight gains/week of the supplemented groups were more than twice those

of the corn grits group. Urinary excretions of nicotinic acid and N-methyl nicotinamide, calculated as nicotinic acid, accounted for 70–77% of the daily supplements fed. The excretion of rats on the corn grits diet was very low. The excretion levels of animals receiving 3-pyridine methanol tartrate were parallel to those receiving nicotinic acid whereas those receiving nicotinamide excreted more N-methyl nicotinamide and less nicotinic acid. Levels of the phosphopyridine nucleotides measured on the whole blood, serum, red and white cells of well nourished human beings are illustrative of the applicability of this microtechnique to the study of quantities of blood obtainable from the finger.

Blackman reaction velocity constant in continuous and intermittent photosynthesis: a re-evaluation of the significance of 0.01 second dark times. DEAN BURK, JEROME CORNFIELD* AND VERNON RILEY.* *Natl. Insts. of Health, Public Health Service, FSA Bethesda, Md.*

With intermittent illumination of equal time periods (Δ) of darkness and of high light intensity, the limiting period of $\Delta \sim 0.01$ second required (Warburg, 1919) for maximum improvement (y) in photosynthetic yield/unit of light is simply a mathematical expression of approach to unity of the term $y = (1 - e^{-k\Delta})/k\Delta$, where k, the Blackman reaction rate constant, is of the order of several reciprocal minutes, equivalent to Willstatter's values for chlorophyll turnover number in continuous illumination at high light intensity and high CO_2 pressure. In flashing light experiments, where the dark periods are much longer than the light periods, the dark times of ~0.01 second commonly reported for maximum photosynthetic yield/light flash do not provide any simple arithmetic measure of k because the light provisions/flash were too low by orders of magnitude (e.g. EMERSON AND ARNOLD, 1932–3; WELLER AND FRANCK, 1941; TAMIYA, 1949). With sufficiently high light absorption/flash, we have observed dark times of, not ~0.01 second, but of the order of some seconds, with low quantum requirement, and a photosynthetic unit of the order of one chlorophyll molecule. Thus, the various explanations of the reported, very low yield ratio "one molecule O_2 produced/2500 molecules chlorophyll/flash," in terms of a large photosynthetic unit, or insufficient stabilizing catalyst B (inefficient back reaction), are rendered superfluous. This reappraisal of the significance of dark times in flashing light follows from inevitable, heretofore unregarded, mathematical consequences of intermittency (cf. *Scientific Monthly* 73: 213, 1951).

Role of coenzyme A in acetaldehyde oxidation. ROBERT MAIN BURTON (introduced by E. R. STADTMAN). *Sect. on Cellular Physiology, Natl. Heart Inst., Natl. Inst. of Health, Bethesda, Md.*

Extracts of *C. kluyveri* catalyze the phosphate dependent oxidation of acetaldehyde to acetyl phosphate (*J. Biol. Chem.* 180: 1095, 1949). With aged suspensions of dried cells, it was demonstrated that CoA, DPN (or TPN), and a sulfhydryl compound are needed for this conversion. The ratio of activities with DPN and TPN varies with different enzyme preparations suggesting that 2 acetaldehyde dehydrogenases are present with different pyridine nucleotide specificity. Ammonium sulfate fractionation of second extracts of dried cells gives 2 protein fractions (A and B), which are required for the formation of acetyl phosphate from acetaldehyde. Fraction A catalyzes the reaction *1*): acetaldehyde + CoA + DPN $\rightleftarrows$ acetyl-CoA + DPNH + H^+; fraction B can be replaced by purified phosphotransacetylase which catalyzes reaction *2*): acetyl-CoA + Pi $\rightleftarrows$ acetyl-P + CoA. The net result is reaction *3*): acetaldehyde + DPN + Pi $\rightleftarrows$ acetyl-P + DPNH + H^+. *Reaction 1* has been studied spectrophotometrically by the reduction of DPN in the presence of acetaldehyde and CoA and by the oxidation of DPNH by acetyl-CoA. *Reaction 3* has been measured by the further reduction of DPN when orthophosphate and phosphotransacetylase were added to the components of *reaction 1* and more directly by the formation of acetyl phosphate from acetaldehyde in the presence of fraction A, phosphotransacetylase, DPN, CoA, and inorganic phosphate. The reversal of *reaction* 3 was studied by DPNH oxidation in the presence of acetyl phosphate, CoA, phosphotransacetylase, and fraction A.

Possible origins of the methoxyl carbon of lignin in barley (*Hordeum vulgare*). RICHARD U. BYERRUM AND JOHN H. FLOKSTRA (introduced by ROBERT M. HERBST). *Kedzie Chemical Lab., Michigan State College, East Lansing.*

In order to ascertain the possible metabolic origin of the methoxyl carbon of lignin, each of 24 barley plants (*Hordeum vulgare*), 30 days old, was grown in an inorganic nutrient solution containing 2.01×10^{-5} moles of D-L methionine with C^{14} in the methyl carbon and having a total activity of 3.6×10^5 counts/minute. After a 7 day growing period lignin was isolated from the plants, from which the roots were removed, and was found to have a specific activity of 385 cpm/mg. To test the supposition that the radioactive carbon was in the methoxyl group, the lignin was demethylated using HI. The methyl iodide formed with this reagent was reacted with triethyl amine to give triethyl methyl ammonium iodide which could be isolated and counted for radioactivity. Ninety per cent of the radioactivity of the lignin

was recovered in this quaternary salt. The lignin contained 2.3% nitrogen which might include some radioactive methionine. A sample of lignin was therefore oxidized to syringaldehyde and vanillin, using nitrobenzene in a bomb. These aldehydes were isolated as the 2,4 dinitrophenylhydrazones and were found to have the same order of radioactivity as the original lignin. 1.99×10^{-5} moles of radioactive sodium formate containing 7.2×10^{5} cpm total activity were fed to each of 40 barley plants in the same way as was the methionine. Lignin isolated from these plants had a specific activity of 29 cpm/mg. and the triethyl methyl ammonium iodide isolated after demethylation of this lignin contained 68% of the lignin's radioactivity.

Some aspects of the metabolism of methionine. E. S. CANELLAKIS (introduced by HAROLD TARVER). *Div. of Biochemistry, Univ. of California, Berkeley.*

Methionine forms methyl mercaptan upon incubation with rat liver mitochondrial preparations as shown in experiments in which methionine-$C^{14}H_3$ and methionine-S^{35} were used. The methyl mercaptan formed was bound to the mitochondrial proteins by —S—S— bonds and could be removed from the protein by reducing agents such as monothioethylene glycol. It was identified in the form of suitable derivatives with the aid of carrier methyl mercaptan. D-methionine-$C^{14}H_3$ and L-methionine-$C^{14}H_3$ formed methyl mercaptan at equal rates, but α-keto-β-methiolbutyric acid-$C^{14}H_3$ formed methyl mercaptan at a rate more than 3 times that of methionine-$C^{14}H_3$. Therefore it is probable that the formation of methyl mercaptan from methionine proceeds via the corresponding keto acid. Methyl mercaptan-$C^{14}H_3$, upon *in vivo* administration to rats, was an effective source of methyl groups, the carbon of the methyl group being found in the methyl groups of choline, in creatine-creatinine and in the methyl group of methionine. It was also found in the β-carbon of serine. Methyl mercaptan-S^{35} when, similarly administered, was found bound to the liver proteins via —S—S— bonds. The label could not be recovered in the sulfur of methionine nor in that of cystine.

Reversible binding of iodine by soluble proteins. R. KEITH CANNAN. *Dept. of Chemistry, New York Univ. College of Medicine, New York City.*

Proteins are known to remove small amounts of iodine from solution by iodination and by reduction to iodide. We have observed that much larger amounts are bound by proteins in a form which is extractable with organic solvents and titratable with thiosulfate. The bound iodine exhibits an absorption band at 360 mμ similar to that of the tri-iodide ion. The reversible binding follows a simple Langmuir isotherm. A number of proteins have shown a maximum combining capacity of about 0.5 Eq. iodine/atom of protein nitrogen, i.e. about 0.7 g/g. protein. Complex formation is competitively inhibited by iodide and by bromide but not by chloride. Under suitable conditions of pH and of concentration of iodine the iodine-protein complex is completely precipitated from solutions as dilute as 0.02% protein. Under standard conditions the amount of protein in the precipitate may be estimated by titration of the iodine by thiosulfate. In some cases the precipitated protein redissolves in water after removal of the iodine. In others it remains insoluble. Stepwise titration with iodine of a heterogeneous protein such as gelatin effects a separation into fractions of graded molecular size ranges. The synthetic polymer polyvinyl pyrollidone reacts with iodine in a manner very similar to that described above but gives a much more distinctive color than do proteins.

Effect of various ions and co-factors on the respiration of rabbit thymus cells. MELCHIOR D. CARINO AND IRVING R. SHERMAN (introduced by WILLARD A. KREHL). *Tumor Research, Hines Veterans Administration Hosp., Hines, Ill.*

To implement the study of thymus cell physiology studies were made as to the most suitable medium in which to suspend these cells for use in the Warburg apparatus. Media of varying ionic composition were compounded. To these were added thiamin, niacin, riboflavin and thyroxin, singly and in combination. Glucose was used as substrate for these studies. The cells were washed in the medium in which they were to be used except that the co-factors were absent in the wash solutions. It was found that there were great differences in the ability of thymus cells to respire in different media. The optimum medium for the utilization of glucose seems to be Ringer's phosphate with calcium absent, an excess of magnesium, and the addition of niacin and thiamine. Under certain circumstances the other co-factors acted as inhibitors.

Oxidative reactions in the propionic acid fermentation. S. F. CARSON AND EUGENE A. DELWICHE (introduced by J. W. FOSTER). *Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.*

Previous studies (DELWICHE AND CARSON, unpublished) on the propionic acid fermentation have demonstrated that *Propionibacterium pentosaceum* contains enzymes which are typical of a tricarboxylic acid cycle. The presence of aconitase and isocitric dehydrogenase was shown by the conversion (in cell juices) of citrate to α-ketoglutarate with the uptake of one-half mole of oxygen/mole of citrate utilized; α-ketoglutarate was isolated and identified. A wide variety of sub-

strates were oxidized with the uptake of molecular oxygen; addition of coenzyme A accelerated oxidations by acetone-dried cells. Present studies demonstrated that acetone-dried cells were capable of carrying out the following reaction: acetate + oxalacetate → citrate. Isotope experiments were performed on 'resting cell suspensions' (aerobically grown cells), with labeled acetate, ethanol and pyruvate. Citrate, α-ketoglutarate, succinate, pyruvate, propionate, malate and acetate were isolated, purified and degraded to individual carbon fragments. The results clearly fall in line with predictions based upon current concepts of the tricarboxylic acid cycle; for example, with ethanol-2-C^{14} as substrate in the presence of an arsenite block, the α-ketoglutarate which was isolated and degraded was found to contain 93% of its labeling in the Δ position (C-4). Incidentally, this distribution of labeling is an excellent example of the Ogston effect. Experiments with acetate-2-C^{14} and pyruvate-2-C^{14} have confirmed and extended these observations.

Distribution and properties of uric acid riboside. CHARLES E. CARTER AND JOSEPH L. POTTER.* *Dept. of Medicine, School of Medicine, Western Reserve Univ., Cleveland, Ohio.*

Uric acid riboside (UAR) was first isolated by Newton and Benedict from beef erythrocytes and, subsequently, from beef liver by Falconer and Gulland. Application of chromatographic procedures which separate UAR from other ribosides and uric acid, to the isolation and analytical determination of this compound, has resulted in disagreement with pre-existing data. The compound has been found only in beef erythrocytes and the spectrum reported by Falconer and Gulland, which was practically identical with that of 9′ methyl uric acid, differs considerably from that of the compound we have prepared. UAR develops color in the orcinol reagent at a rate slower than that of other purine ribosides and is 20% hydrolyzed in 1N HCl at 100°C. in 1 hour. The compound is resistant to mammalian nucleosidases, but is cleaved to uric acid and ribose by bacterial extracts. Proof of structure by chemical or enzymatic synthesis from ribosides of known structure has not been achieved. The relative resistance to acid hydrolysis and ultraviolet absorption data, though suggestive of a nucleoside structure involving the 3′ or 7′ position of the purine ring, may be explained by resonance stabilization conferred upon a 9′ purine riboside by hydroxyl substitution in the 8′ carbon.

Effect of x-ray on salt and water balance in the rat. W. O. CASTER (introduced by CYRUS P. BARNUM). *Physiological Chemistry Dept., Univ. of Minnesota, Minneapolis.*

In the week following total body irradiation with an LD_{50} dose of 160 kv. x-ray, rats lose 10–20% of their body weight. The water intakes increase some 2- to 3-fold and the excretion values become very large. A group of 20 rats was maintained on metabolism for a 3-week control period, irradiated with 700 r of x-ray, and studied for an additional 2 weeks. Sodium, potassium and chloride balance studies were carried out during the entire period. The potassium balances promptly became negative following irradiation; but only 20% of the weight loss during the first 48 hours could be explained in terms of loss of intracellular material. The sodium balances did not decrease as quickly but did become significantly negative. The chloride balance was positive immediately after x-irradiation but became negative after 4–6 days. During the first week following irradiation there was twice as much weight lost as could be accounted for in terms of the negative cation balances. When expressed as a percentage of body weight there was essentially no change in blood volume, extracellular space or total body water following x-ray. Serum sodium, chloride and total base (by conductivity) levels increased slightly but significantly during the postirradiation period. The urinary excretion of desoxycorticosterone activity following irradiation was not significantly different from control values.

Effect of traces of L-histidine upon the growth of mice fed D-histidine or imidazolelactic or urocanic acid. D. R. CELANDER* AND CLARENCE P. BERG. *Dept. of Biochemistry, College of Medicine, State Univ. of Iowa, Iowa City.*

Preliminary evidence has indicated that mice fed a histidine-deficient basal diet supplemented with D-histidine fail to grow when the vitamin B complex is supplied as a mixture of the purified components, but grow slowly when a yeast vitamin concentrate is substituted (*Federation Proc.* 1: 283, 1942). This has been confirmed. The ingestion of 1 mg. of L-histidine/day permits slow growth to occur on the D-histidine diet containing the purified vitamin mixture. Additions of hydrolysates of the yeast vitamin concentrate also permit growth to occur, unless they are first freed of histidine. The growth-promoting effect of the yeast vitamin concentrate therefore seems to be attributable to its L-histidine content. The presence of small amounts of L-histidine is apparently mildly stimulatory, rather than simply additive. No growth occurred on the basal diet containing the usual quantity of yeast vitamin concentrate, the purified vitamin mixture plus the 1 mg. of L-histidine/day, or the purified mixture plus the yeast vitamin hydrolysate. Imidazolelactic acid produced responses similar to those with D-histidine. Urocanic acid showed no capac-

ity to promote growth, even when the diet was supplemented with 1 mg. of L-histidine daily.

Respiratory pigments of metabolising cells. BRITTON CHANCE. *Johnson Research Fndn., Univ. of Pennsylvania, Philadelphia.*

Various spectrophotometric methods now permit the rapid *in vivo* assay of the various types of cytochromes, pyridine nucleotides and flavoproteins of microorganisms: *1*) The visible and Soret bands of the carbon monoxide and other compounds of the cytochrome oxidase of respiring yeast and bacteria have been studied by a new differential method that directly plots the spectra representing the difference between the absorption of the carbon monoxide compound and the reduced form of the oxidase. Yeast cells and heart muscle homogenates both show peaks of cytochrome a_3-CO at 429.5, 540, and 589 mμ. In *Aerobacter aerogenes*, cytochrome a_2 differs markedly from a_3 and, in fact, from hemoproteins in general; it shows no distinctive Soret band in the reduced form, and the Soret band of its carbon monoxide compound (437 mμ) is only one third as intense as the visible band (645 mμ). *2*) In several microorganisms, the spectrum of reduced pyridine nucleotide is also clearly registered and shows varying degrees of a shift towards 325 mμ. This is probably due to the type of protein bond that forms with alcohol dehydrogenase. The technique of Theorell and Chance that permits independent measurement of this type of bound DPNH shows that nearly all the pyridine nucleotide in *Aerobacter aerogenes* is so bound, while in bakers yeast and in *Escherichia coli* (B) very little is so bound. Kinetic differences between this bound pyridine nucleotide and that absorbing at 340 mμ are observed at the onset of anaerobic conditions: the form absorbing at 340 mμ undergoes partial oxidation upon the initiation of anaerobic glycolysis; the form absorbing at 325 mμ does not, and hence its function is probably directly associated with cytochrome.

Incorporation of formaldehyde into phosphorylated sugars. FRIXOS CHARALAMPOUS (introduced by CHARLES HEIDELBERGER). *McArdle Memorial Lab., Med. School, Univ. of Wisconsin, Madison.*

Dialyzed supernatant fractions obtained by high speed centrifugation of rat liver homogenates metabolize formaldehyde anaerobically in the presence of hexosediphosphate or dihydroxyacetone phosphate. The rate of formaldehyde removal is not affected by blocking triose oxidation with iodoacetate and appears not to require known co-factors. With the aid of $C^{14}H_2O$ the products of the reaction have been isolated from the reaction medium by adsorption on Dowex 1. On elution from the column with acid and rechromatographing the eluate on paper, using 2 different solvent systems, the radioactivity is distributed among 3 compounds which yield phosphate on acid hydrolysis. The major activity is confined to a single area. Treatment of the eluate with phosphatase yields radioactive products which are no longer retained on the Dowex 1. Paper chromatography with 3 different solvent systems revealed again 3 distinct radioactive areas; the first area containing 10% of the activity coincides with D-ribose in individual or mixed chromatograms with the authentic compound. The second area contains 7% of the radioactivity and migrates at the same rate as L-arabinose or glucose. The third area containing 80% of the radioactivity occupies a position midway between known pentoses and trioses. An aldotetrose is indicated by the chromatographic character and color reactions. The enzyme system although resembling the aldolase exhibits definite differences from it and tumors are quite low in the present system. Additional data on the condensation of formaldehyde with a phosphorylated triose and sequential reactions will be presented.

Histochemical use of dithizone. HOWARD H. CHAUNCEY AND FABIAN LIONETTI (introduced by BURNHAM S. WALKER). *Dept. of Biochemistry, Boston Univ. School of Medicine, Boston, Mass.*

Dithizone (diphenylthiocarbazone) in acetone solution has been utilized as a stain for leucocytes and erythrocytes. Whereas the erythrocyte presents a homogeneous orange color, leucocytes of various species tested, including human and rabbit, show homogeneous pink cytoplasm with dark granulation and little nuclear stain. The polymorphonuclear cells contain about 10 times as much granulation as mononuclear leucocytes. When ZnO was used with starch as a subcutaneous irritant in the rabbit, the cells obtained from the abcess were similar in detail but much more intensely colored. However, exudates of leucocytes obtained from suspensions in saline of either ZnO or dithizone injected into the abdominal cavities of rabbits stained in no unusual way and appeared like normal cells. Slides prepared from subcutaneous abcesses produced by injection of dithizone-starch mixtures likewise exhibited no intensification of color, probably because of the great insolubility of the dye. Chicken leucocytes did not stain with dithizone. The cytoplasm of chicken erythrocytes resembled human erythrocytes with the nuclei remaining unstained. Only 5% of leucocytes obtained from a human being with chronic myelocytic leukemia showed any tendency to stain. These were grossly comparable to normal cells in detail but yellow in color.

Gastric mucosa which normally exhibited pink color with dithizone increased in color in ZnO-fed mice. A slight increase was observed in kidney sections. Although, owing to the inability to buffer the preparation, the dependence of the staining upon Zn content could only be inferred, the data are consistent with known or predictable Zn levels.

Surface film method as a means for steroid estimation. CHIADAO CHEN AND MARGARET J. SULLIVAN (introduced by ALMA HILLER). *Dept. of Biochemistry, Northwestern Univ. Med. School, Chicago, Ill.*

It is long known that steroid molecules, when spread in a monomolecular layer, give an area of from 30–120 $A^{\circ 2}$, depending upon the type of compound and the force of compression. A number of steroids dissolved in suitable solvents, such as cyclohexane, benzene, and others, were applied on water surface. The areas of the monolayer films formed were measured under different forces of compression. The piston oil method and mica float method were used and the results studied. Some steroids were found not to give monolayer film and some others were soluble in the substratum. The degree of accuracy was satisfactory for steroid quantitation with even very simple apparatus. Considerable information concerning the constitution of unknown steroids has been obtained with a few μg. of the material within a few minutes. It promises to be helpful in quantitation and identification of steroids, particularly of those that cannot be detected by other means in such small quantities. (Work supported by a U.S.P.H. Grant.)

Time relationships of certain renal processes. FRANCIS P. CHINARD. *Depts. of Physiological Chemistry and of Medicine, Johns Hopkins School of Medicine, Baltimore, Md.*

Data have been obtained on the time relationships of certain renal processes in anaesthetized dogs. Test substances are injected 'instantaneously' into the left renal artery, urine is sampled from each catheterized ureter separately for a number of periods of 4–30 seconds' duration (in most experiments a diuretic is administered); in some experiments blood is sampled at intervals of 1–2 seconds from the catheterized left renal vein. The right kidney serves as control. Processes studied and time relationships: *1*) circulation through kidney and renal vein catheter of P^{32} labelled red cells and T-1824—minimum time 3 seconds, most probable time 6 seconds; *2*) antidiuresis with serum albumin—immediate; *3*) diuresis with mannitol—immediate; *4*) mercurial diuresis—5–8 minutes; *5*) inhibition of mercurial diureses by sulfhydryl compounds—5–8 minutes; *6*) most probable time for circuit renal artery through ureteral catheter for inulin, creatinine and ferrocyanide—4–12 seconds longer than appearance time; *7*) secretion of PAH—most probable time is 20–30 seconds longer than most probable time for inulin, creatinine and ferrocyanide. The significance of these findings will be discussed with respect to current concepts of renal physiology. (This work was supported by a grant from the Life Insurance Medical Research Fund.)

Identification of products of lecithin hydrolysis by paper chromatography. DORIS H. CLOUET (introduced by NORMAN S. OLSEN). *Research Lab., Thayer Veterans Administration Hosp. and Biochemistry Dept., Vanderbilt Univ., Nashville, Tenn.*

In the study of phospholipid hydrolysis the dissimilarity of the hydrolytic products hinders the qualitative identification of these products. Paper chromatography offers a technique which is particularly suited to the rapid identification of compounds as unlike as choline and fatty acids. A chromatographic scheme is presented for the separation on filter paper of products of lecithin hydrolysis such as lysolecithin, glycerophosphoric acid, choline, inorganic phosphate, glycerylphosphorylcholine, fatty acids, and lecithin itself, as well as other compounds liable to occur in tissue suspensions such as ATP, hexosediphosphate, serine, and ethanolamine. Among the solvent systems employed the most useful are phenol-water, ethanol-water, and *n*-butanol-morpholine-water. The compounds containing glycerol or hexose in the molecule (as well as compounds containing a hydroxyl and amino group on adjacent carbon atoms, such as serine) have been developed by spraying with periodic acid followed by fuchsin reagent. Inorganic phosphate is visualized by the molybdate-aminonaphtholsulfonic acid color reaction, and choline by the formation of a reduced phosphomolybdate complex. Serine and ethanolamine are developed by spraying with iodine, while the presence of all of the fatty acids is revealed by spraying with an indicator such as bromcresol purple, or by the hydroxamic acid-ferric chloride color reaction. The position on filter paper of the compounds containing phosphorous has been checked by chromatography, and subsequent autoradiography, of hydrolytic products of lecithin containing radiophosphorous.

Activation of creatine synthesis in homogenates of rat liver by folic and glutamic acids. STANLEY COHEN (introduced by FLORIAN CAJORI). *Dept. of Pediatrics, Univ. of Colorado School of Medicine, Denver.*

Previous work in several laboratories has indicated that homogenates of guinea pig liver can synthesize creatine in the presence of guanido-

acetic acid, L-methionine, and ATP. However, liver homogenates of the rat and of several other species are much less active in this respect. With appropriate fortification, homogenates of rat liver can synthesize creatine at a rate comparable to that of homogenates of guinea pig liver. In the combined presence of folic and glutamic acids, preparations of rat liver are capable of synthesizing creatine from guanidoacetic acid, L-methionine, and ATP. The omission of any one of these reagents slows or completely abolishes the synthesis.

Effect of certain naphthoquinones on fat formation and the utilization of 1-C^{14} acetate by *fusarium lini* bolley. R. J. COLEMAN* AND F. F. NORD. *Dept. of Organic Chemistry and Enzymology, Fordham Univ., New York City.*

When *Fusarium lini* was grown in a medium in which acetate served as the substrate, it was found that the mat weights varied linearly with the concentration of acetate in the medium up to 2%. In addition to the increases in mat weights, there was a corresponding increase in the amount of fat formed. The mat weights and the amount of fat formed from acetate are comparable to those when glucose is the substrate. Thus acetate may be considered to be along the pathway in the course of the glucose → fat conversion. However, certain naphthoquinones were shown to appreciably lower the mat weights, amount of fat formed and the conversion coefficients, when added to a medium in which glucose was the sole carbon source. When these same naphthoquinones were added to a medium containing acetate as the carbon source none of these values were significantly lowered when compared to the control. Further studies on the utilization of acetate were carried out with culture media containing 1-V^{14} labeled acetate. In these media a mixed carbon source consisting of acetate and glucose was present. The specific activities of samples of mycelia, which differed in age by 8 days were found to be the same. During this period there was a substantial increase in the mat weights, and since there was no residual glucose in either case, the glucose must have been broken down to smaller units, which were present as such in the medium.

Hydrolysis of growth hormone by crystalline chymotrypsin and carboxypeptidase. PETER G. CONDLIFFE* AND CHOH HAO LI. *Dept. of Biochemistry, Univ. of California, Berkeley.*

Crystalline growth hormone prepared according to Li, Evans and Simpson (*J. Biol. Chem.* 159: 353, 1945; and *Science* 108: 624, 1948) was hydrolysed with crystalline chymotrypsin at pH 8.5 and 25°C. with an enzyme to hormone ratio of 1:200. After 2 hours of hydrolysis, the 5% trichloracetic acid soluble material increased from 1–2% to 30%, and approximately 8–12 peptide bonds were broken. As shown in the table, assay of the hydrolysate, by the tibia test, showed that 50% of the original activity was retained up to 34% hydrolysis. Electrophoretic examination of the hydrolysates revealed the presence of a new major component, which had a different mobility from that of growth hormone. Moreover, no component was observed identifiable in electrophoresis as unhydrolysed native growth hormone.

EXPER. NO.	TIME OF INCUBATION	HYDROLYSIS BY CHYMOTRYPSIN	ACTIVITY REMAINING
	hr.	%	%
Control	24	0	100
107A3	1	16	100
114A1	1.75	25	50
112A	2.25	34	50
110A4	3	39	0

When growth hormone was reacted with crystalline carboxypeptidase, no significant reduction in biological potency could be demonstrated after 24 hours' incubation at pH 8.5, 25°C. with an enzyme to growth hormone ratio of 1:50. Paper chromatographic analysis of the trichloracetic acid soluble material from such hydrolysates showed that serine, alanine and phenylalanine were released from the hormone in significant amounts, while traces of glutamic acid, leucine, threonine, glycine, arginine, histidine and lysine were present in some experiments. Thus, it may be concluded that COOH terminal end groups are not essential for the biologic potency of growth hormone.

Influence of starvation and of pyruvate administration upon the utilization of radioacetate in the intact rat. JOHN G. CONIGLIO,* CARL E. ANDERSON AND C. S. ROBINSON. *Dept. of Biochemistry, Vanderbilt Univ. School of Medicine, Nashville, Tenn.*

A tracer dose of sodium acetate-1-C^{14} was administered intraperitoneally to 4 groups of rats, *1*) nonfasted, given acetate alone; *2*) nonfasted, given acetate plus 200 mg. inert sodium pyruvate; *3*) starved 72 hours, given acetate alone; and *4*) starved 72 hours, given acetate plus pyruvate. Expired carbon dioxide was collected for 24 hours, and the animals were sacrificed at the end of this time. Lipids of the various tissues were extracted, fractionated, and hydrolyzed, and the resulting fatty acids were assayed for radioactivity. The animals of *1* expired 50% of the administered activity in 46 minutes. In *2* this time was increased to 80 minutes. Starvation alone *3* raised this value still more. Differences between *3* and *4* were inconclusive. Pyruvate administration to *2* did not decrease the enrichment of the biosyn-

thesized fatty acids (compared with *1*); in fact, the data suggest a greater incorporation of activity in the fatty acids of *2* than in *1*. All starved rats showed great depletion of carcass fat with a corresponding decrease in total fatty acid radioactivity. There were no significant differences in total fatty acid C^{14} incorporation between *3* and *4*. The ratio of the specific activities of fatty acids of the acetone-soluble and acetone-insoluble fractions of mesenteric and subcutaneous lipids was variable. Consistent data were obtained for liver, intestine, and intramuscular fat (ratios of 1.0, 0.7, and 1.2 to 2.4, respectively).

Metabolic alteration of 4-amino-5-imidazolecarboxamide in tumor-bearing mice. GAYLORD M. CONZELMAN, JR.,* H. GEORGE MANDEL* AND PAUL K. SMITH. *Dept. of Pharmacology, George Washington Univ. School of Medicine, Washington, D.C.*

Studies have been made to determine the metabolic pathway of a purine precursor, 4-amino-5-imidazolecarboxamide, in CAF_1 mice bearing sarcoma 37. The compound labeled in the C_4 position was synthesized from $NaC^{14}N$ in a 45% yield. Excretion studies after subcutaneous administration of 500 mg/kg showed that two thirds of the radioactivity was excreted in the urine within 7 hours. Two radioactive urinary metabolites have been separated by countercurrent distribution and paper chromatography, one of which is indistinguishable from the original compound. Only a trace of radioactivity has been recovered in the expired CO_2. Divided administrations of the daily dose of the compound to adult mice increased the incorporation. Because the substance was found to be absorbed readily from the gastrointestinal tract, it was mixed in the diet for 6 days at a total dose of 500 mg/kg. Twenty-four hours after the termination of the radioactive diet, the animals were sacrificed, the liver, kidneys, spleen and thymus removed and pooled and the tumor excised. The protein fraction showed negligible activity. Isolation and degradation of RNA and DNA by modifications of existing methods indicated that most of the activity was in the adenine and guanine fractions. The specific activity of the purines, after purification by ion exchange on Dowex 50 and direct plating on glass discs, was determined in a gas-flow proportional counter. There was no evidence of incorporation into the pyrimidine fraction.

Conversion of 1-C^{14}-mannose and 1-C^{14}-glucose to glycogen in the intact rat. MARGARET COOK (introduced by ERNEST B. BUEDING). *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

The epimeric sugars, 1-C^{14}-mannose and 1-C^{14}-glucose, were administered to fasted rats. The intraperitoneal route was selected because of the poor absorption of mannose from the intestine. Nonisotopic glucose was given by stomach tube in both experiments to enhance glycogen formation. The resulting liver and muscle glycogen were isolated, hydrolyzed to glucose, and the isotope distribution in the hexose chain determined. In both the glucose and mannose experiments, in muscle as well as liver glycogen, 80–90% of the activity isolated in the glycogen resided in carbon one. The balance of the activity was distributed more or less uniformly among the remaining carbons of the hexose chain. The specific activity of the glycogen fractions and the percentage of fed activity recovered in these fractions as well as in the respiratory CO_2 was the same for both glucose and mannose. The specific activity of the muscle glycogen was about half that of the liver glycogen in both cases. It is concluded that a direct conversion of mannose to glucose, not involving chain cleavage, occurs in mammalian tissue.

An insoluble hexokinase from calf brain cortex. ROBERT K. CRANE AND A. SOLS (introduced by EARL W. SUTHERLAND). *Dept. of Biochemistry, Washington Univ. School of Medicine, St. Louis, Mo.*

As much as 90% of the total hexokinase activity of fresh homogenates of calf brain cortex prepared in dilute phosphate buffer can be sedimented by centrifugation at $15000 \times g$; the remaining 10% is found in the supernatant liquid. The particulate fraction which sediments between $800 \times g$ and $3000 \times g$ has a specific activity (μM of glucose used/mg. of protein) 6–8 times that of the original homogenate. This fraction when washed several times with phosphate buffer is essentially free of phosphohexokinase activity. Whereas the rate of glucose phosphorylation using crude homogenates or extracts is linear with time, the rate with this purified fraction falls off with time, presumably due to accumulation of glucose-6-phosphate. This is deduced from the facts that added phosphohexokinase prevents the fall in rate with time and that added glucose-6-phosphate is strongly inhibitory. Chemical analysis shows this preparation to contain approximately 37% lipid, of which some 60% is phospholipid and about 1% nucleic acid. Treatment with a commercial lipase releases inactive protein and results in a 4 to 5-fold additional purification. Further work on the nature and purification of this enzyme is in progress.

Incorporation of labeled dimethylethanolamine in phospholipides of isolated liver tissue. MARIETTA CROWDER AND CAMILLO ARTOM. *Dept. of Biochemistry, Bowman Gray School of Medicine, Winston-Salem, N.C.*

Slices or homogenates of rat liver were incubated with C^{14}-methyl labeled dimethylethanolamine (DME). Considerable amounts of the compound were incorporated directly into the phospholipides without previous methylation to choline. This finding parallels those of previous experiments *in vivo* (*Federation Proc.* 8: 180, 1949). In oxygen the apparent uptake of DME by the lipides is greater with liver slices from rats on methyl-deficient diets than from rats on a stock diet. Homogenization reduces but does not suppress this uptake. With both slices or homogenates the amounts of DME incorporated in the lipides are greater in nitrogen than in oxygen. The process is markedly inhibited by betaine added *in vitro*, less markedly by methionine. The above findings will be compared with those previously reported for the incorporation of labeled phosphate (*J. Biol. Chem.* 193: 473, 1951) and labeled choline (*Federation Proc.* 10: 157, 1951) into the phospholipides of isolated liver tissues. (Aided by the Markle Foundation and by the U. S. Atomic Energy Commission, Contract No. AT-(40-1)-288, Title II).

Liver and muscle glycogen as influenced by alloxan. Owen S. Cullimore,* Phoebe J. Bradshaw,* N. R. Blatherwick and Eliot F. Beach. *Biochemical Lab., Metropolitan Life Insurance Co., New York City.*

Liver and muscle glycogen have been determined in albino rats 6, 18, 30 and 48 hours and at 4 weeks after alloxan injection. Alloxan injection was preceded by a 96-hour fast and followed by *ad libitum* feeding of Purina Dog Chow. During the 6-hour period before taking tissues for analysis a solution of glucose and peptone was given by mouth, the quantity being based upon body weight. Tissues were frozen *in situ* under amytal anesthesia before removal for analysis by the anthrone method. Unalloxanized controls were used for comparison. Nonfasted normal rats show a sex difference in postprandial liver glycogen, the males having 2000–4000 and the females 850–2000 mg.%. Liver glycogen is greatly reduced by fasting 96 hours but after 18 hours refeeding is stored at above normal levels (5000–8000 mg.%.) Reaction to alloxan changes the pattern of liver glycogen storage. With strong hyperglycemia 18 hours after alloxan liver glycogen may go as low as 250 mg.%. At 30 hours when hypoglycemia occurs liver glycogen may rise to 6000 mg.%. Re-establishment of the hyperglycemia at 48 hours is accompanied by liver glycogen as low as 60 mg.%. At this time also muscle glycogen has been found to fall to 250 mg.%, about one third of its normal level. In diabetes established for a period of 4 weeks the liver glycogen is considerably reduced (300–1100 mg.%.)

Treatment of granulocytopenia in folic acid-deficient rats with adenine. Floyd S. Daft. *Natl. Insts. of Health, Bethesda, Md.*

It has previously been reported from this laboratory that rats receiving purified diets containing 'sulfasuxidine' (succinyl sulfathiazole) or large amounts of iron or diets low in protein show growth failure, leucopenia, granulocytopenia and anemia. These deficiency signs are prevented or corrected in whole or in part by folic acid. In view of the success attained by others in substituting adenine and thymine for folic acid in media for bacteria and in treating pratients with macrocytic anemias with thymine, these compounds have been tested therapeutically in our granulocytopenic rats. When adenine hydrochloride and thymine were given together in the diet, each at a level of 1%, marked leucocyte and granulocyte response were obtained. Growth was not resumed, however, the animals usually remained or became anemic, and kidney damage was observed. Most or all of the activity appeared to be due to the adenine. It has previously been shown in this laboratory that the deficiency syndrome in rats receiving low protein diets could be prevented by a mixture of methionine, tryptophane and threonine. Treatment of granulocytopenic animals with this mixture was usually not effective, however, unless histidine or folic acid or PABA was given in addition. Considerable success in treatment has now been accomplished with a mixture of methionine, tryptophane and threonine with adenine, adenosine or yeast adenylic acid. One possible explanation of these results is that folic acid-deficient rats are unable to synthesize amounts of adenine needed for white cell formation.

Prevention of certain B vitamin deficiencies with ascorbic acid or antibiotics. Floyd S. Daft and Klaus Schwarz. *Natl. Insts. of Health, Bethesda, Md.*

It was reported last year that ascorbic acid at high levels (2% of the diet) or antibiotics prevented or delayed the deficiency signs of certain B vitamins in rats. Results with ascorbic acid in pantothenic acid-deficient diets were reported in detail. Rats placed at weaning on diets devoid of pantothenic acid or riboflavin developed deficiencies and died as usual but litter-mates receiving diets identical except containing 2% of ascorbic acid or 20 mg.% of aureomycin have now been under observation for 1 year. Most of the animals attained weights in excess of 200 gm. in 2–4 months; after 1 year several males weighed over 500 gm. Not all animals were protected by ascorbic acid or aureomycin. A few died during the observation period with typical deficiency signs and some survivors on the ribo-

flavin-deficient diets showed severe skin lesions. Most rats on the pantothenic acid-deficient diets, however, and some on the riboflavin-deficient diets appeared normal when sacrificed after 1 year. No gross pathology was detected except a mottled appearance of livers of a few pantothenic acid-deficient animals, possibly due to fatty infiltration. In addition, experiments of shorter duration with chloromycetin in pantothenic acid-deficient diets have given similar results. In preliminary studies, 5% ascorbic acid in thiamine-free diets prevented or delayed the onset of deficiency signs.

Peptidase action on peptides containing proline and hydroxyproline. NEIL C. DAVIS AND ELIJAH ADAMS (introduced by EMIL L. SMITH). *Lab. for Study of Hereditary and Metabolic Disorders, Univ. of Utah, Salt Lake City.*

Several new peptides and derivatives containing proline and hydroxyproline were synthesized in order to delineate further the specificity of certain peptidases. Prolinase of swine kidney which hydrolyzes L-prolylglycine and similar dipeptides containing proline and hydroxyproline is activated by Mn^{++} and, to some extent, by Cd^{++} (NEUMAN AND SMITH *J. Biol. Chem.* 193: 97, 1951). It has now been observed that the hydrolysis of L-prolinamide, hydroxy-L-prolinamide and hydroxy-L-prolylglycylglycine by kidney extracts is similarly activated. These findings suggest that prolinase is an iminopeptidase which requires in the substrate a free imino group but does not require a free carboxyl group. The tripeptidase of horse erythrocytes which is not activated by metal ions (substrate, triglycine) does not hydrolyze glycyl-L-prolylglycine and appears to have a slow action on hydroxy-L-prolylglycylglycine. Erythrocyte prolidase (substrate, glycyl-L-proline) activated by Mn^{++} and inhibited by Cd^{++} has no action on glycyl-L-prolylglycine or on glycylglycyl-L-proline and appears to be a dipeptidase specific for a peptide bond involving the imino group of L-proline or hydroxy-L-proline.

Metabolism of ribose-5-phosphate and ribulose-5-phosphate. G. DE LA HABA* AND E. RACKER. *Dept. of Microbiology, New York Univ. College of Medicine, New York City.*

Crude extracts of bakers' yeast catalyse the formation of triose phosphate from phosphate esters of ribose and ribulose. The appearance of triose phosphate was followed spectrophotometrically with reduced DPN and an enzyme preparation from rabbit muscle containing glycerophosphate dehydrogenase and triose phosphate isomerase. Fractionation of the yeast enzyme resulted in the resolution of 2 enzymatic steps in the breakdown of ribose-5-phosphate. A marked lag period was observed in the formation of triose phosphate with the purified enzyme (enzyme II) when ribose-5-phosphate was used as substrate. This lag was completely eliminated if ribose-5-phosphate was first incubated with another fraction (enzyme I) obtained from yeast, which alone did not catalyse triose phosphate formation from ribose-5-phosphate. The purification of these enzymes by fractionation with acetone, alcohol, alumina gel and ammonium sulfate will be reported. Purified enzyme II (splitting enzyme) which has negligible activity with ribose-5-phosphate as substrate is capable of splitting ribulose-5-phosphate to triose phosphate. No accumulation of a two-carbon fragment with properties of glycolaldehyde could be detected. However, formation of a sugar phosphate identified as hexose monophosphate by 3 independent enzymatic tests has been demonstrated with partially purified preparations. On further purification the formation of hexose monophosphate from ribose-5-phosphate can be eliminated and restored to a system consisting of enzyme I and II by the addition of a third enzyme fraction from yeast.

In vitro incorporation of radioactive phosphate into rabbit reticulocytes. H. A. DELUCA AND T. D. LOVEGROVE (introduced by A. B. MACALLUM). *Dept. of Biochemistry, Univ. of Western Ontario, London, Ontario, Canada.*

Suspensions of rabbit red blood cells containing a high percentage of reticulocytes were obtained by the method of successive daily bleedings. When radioactive phosphorus in the form of inorganic phosphate was added *in vitro* to such suspensions, the cells took up the labeled phosphate more rapidly than red cell suspensions not containing a high percentage of reticulocytes. The more rapid incorporation of radioactive phosphate into the cells of a rabbit subjected to daily bleedings was not due to the accompanying lipemia. When Krebs-Ringer-phosphate was substituted for plasma as the suspending medium, the rate of penetration of labeled phosphate into mature red cells or mixtures of red cells and reticulocytes was slower, but when Krebs-Ringer-bicarbonate was substituted, the penetration was more rapid. The rate of uptake was decreased by the presence of cyanide (0.005 M) in the suspending medium.

Reduction of nitrite and nitrate ions by preparations obtained from higher plants. C. C. DELWICHE (introduced by P. K. STUMPF). *Div. of Plant Biochemistry, Univ. of California, Berkeley.*

Preparations obtained by grinding tissues of higher plants in isotonic glucose-phosphate solutions, filtering through cheese cloth and centrifugal fractionation between 1,000 and 20,000 *g*.

are capable of converting N^{15} labeled KNO_3 or KNO_2 to reduced forms from which NH_4^+ can be recovered containing the isotopic nitrogen. Varying degrees of activity were found in all tissues investigated including tobacco leaves, spinach leaves, mung bean seedlings, barley roots and tops, radish roots, squash seedlings, and sugar beet seedlings. Optimum activity is obtained with the preparation when dialyzed plant juice, citrate or other Krebs intermediate and a combination of co-factors are added. Fractionation of co-factors is being carried out. System is inhibited by $Cu(10^{-3}$ M), AsO_4 and iodoacetamide. Boiling of the preparation inactivates.

Effect of fortification and inhibitors on phenol conjugation by rat liver homogenate and fractions. R. H. DE MEIO. *Dept. of Biochemistry, Jefferson Med. College, Philadelphia, Pa.*

Rat liver homogenate conjugates phenol with sulfate to a slight extent (10%) if fortified with succinic acid; less conjugation is observed if only AMP is added, but when both are added together from 40–80% of the phenol is conjugated. If ATP is substituted for AMP, an increase in the activity of the preparation is observed; a further increase takes place by addition of 10^{-5} M cytochrome *c* (100% conjugation). The conjugation of phenol is completely inhibited by 3×10^{-5} M methylol gramicidin and 10^{-2} M azide. 0.02 M sulfate has no effect. Addition of 0.003 M ATP to the homogenate (in anaerobiosis), to the acetone powder, or to the mitochondrium-free supernate results in low activity (5% conjugation). Fractionation of the liver homogenate by differential centrifugation shows that maximum activity is obtained when the mitochondrial and mitochondrium-free supernate fractions are mixed (the individual fractions are inactive). Liver acetone powder shows very low activity (4% conjugation) when mixed with mitochondria and inhibits conjugation when added to the mitochondria plus mitochondrium-free supernate preparation. (This work was supported in part by contract NONR 229 (00) with the Office of Naval Research.)

Effects of chemical sterilizing agents on serum albumin. MARSHALL E. DEUTSCH (introduced by GEORGE H. MANGUN). *Henry Ford Hosp., Detroit, Mich.*

In the course of a screening program, it was noted that viricidal carbonyl compounds added to human blood plasma caused an increase in the electrophoretic mobilities at pH 8.6 of the plasma proteins. This effect was produced by glyoxal, methyl vinyl ketone, mucobromic acid, acrolein, pyruvic aldehyde, chloroacetaldehyde, diacetyl ethylene, and ethyl chloroformate, among other compounds. The progressive changes in electrophoretic patterns which were seen were first disappearance of the $alpha_1$ component, then blunting of the albumin peak, and next a general blunting of the pattern which was associated with and followed by an increase in the proportion of a component with the mobility of beta-globulin or a slightly greater mobility. These observations suggested that the reactions of these compounds with plasma proteins might primarily be with the basic groups of the proteins and that cross-linking also occurred. However, when solutions of bovine serum albumin were treated with the same concentrations of these reagents at a pH (6.65) and ionic strength similar to that of plasma, the electrophoretic mobility of the albumin *decreased*. This effect was reduced or even reversed when the albumin was treated in the presence of glycine at a molar concentration about equal to the amino acid concentration of postabsorptive plasma. The results of titration of the treated albumin suggest that the effect of glycine on electrophoretic mobility under these conditions was primarily due to its inhibition of cross-linking between albumin molecules.

Effect of folic acid and citrovorum factor on N′-methylnicotinamide excretion in the rat. L. S. DIETRICH,* W. J. MONSON* AND C. A. ELVEHJEM. *Dept. of Biochemistry, Univ. of Wisconsin, Madison.*

Growing rats fed a purified ration containing ample amounts of all B vitamins were able to efficiently methylate and excrete, as N′-methylnicotinamide, daily injected doses of nicotinamide as high as 80 mg/kg. body weight. On the other hand rats made folic acid deficient by the feeding of a folic acid free purified ration containing 2% sulfasuxidine excreted significantly lower levels of N′-methylnicotinamide. A single injection of 10 μg. or less of folic acid increased the excretion of N′-methylnicotinamide in the folic acid deficient rat to a level equivalent to that of the normal rat. This response lasted in all cases longer than 8 days. However, a single injection of synthetic citrovorum factor (Leucovohin, Lederle) equivalent to 10 μg. of the free acid was without effect. The administration of 150 μg. of Leucovohin produced a marked but temporary response which completely disappeared within 48 hours. The significance of these observations will be discussed.

Mucopolysaccharides of glycoproteins of human serum. ZACHARIAS DISCHE AND MARTHA OSNOS.* *Dept. of Biochemistry, College of Physicians and Surgeons, Columbia Univ., New York City.*

Two polysaccharide fractions representing 90% of total polysaccharide of glycoproteins were prepared from human serum as follows. Serum proteins precipitated with 90% ethanol were sus-

pended for 20 hours at 24° in a 5% solution of KOH in 80% ethanol. About 90% of the protein dissolved the insoluble residue, containing about 70% of the total protein polysaccharide, washed with the solvent dissolved in water (Fraction I). The clear supernatant of the original residue was left for 24 hours at 56°. An oily layer separated which washed with solvent and dissolved in water contained about 15% of the total protein polysaccharide (Fraction II). Methylpentose, hexose and hexosamine were found and determined in both fractions by their reactions with cysteine in H_2SO_4 and with indole in HCl. In Fraction I equivalent amounts of galactose and mannose were found and ratios of galactose: hexosamine: methylpentose of 0.7 and 9, respectively. The ratios remained unchanged after fourfold reprecipitation with 5% KOH in 80 or 67% ethanol, while the ratio total sugar N_2 rose to 4 and remained unchanged after further reprecipitation. Methylpentose was split off the polysaccharide chain by short hydrolysis with 5% TCA at 90°. These facts suggest that the glycoproteins of the serum contain a mucoid resembling in structure blood group substances and attached to proteins by weak alkalilabile bonds. Fraction II, like the acid soluble mucopolysaccharide of the serum, contains galactose, mannose, methylpentose, hexosamine in proportions different from those in Fraction I and more firmly bound to protein and a third unidentified hexose.

A soluble pyruvate—α ketobutyrate dehydrogenase system from *Streptococcus faecalis*. M. I. DOLIN* AND I. C. GUNSALUS. *Dept. of Bacteriology, Univ. of Illinois, Chicago.*

Extracts have been obtained from dried cells of *Streptococcus faecalis*, strain 10Cl, which catalyze the dismutation of 2 molecules of α-keto acid to the acyl phosphate, α-hydroxy acid and CO_2 (Krebs' dismutation). With pyruvic acid as substrate there is a concomitant formation of acetoin, whereas with ketobutyrate as substrate, no acyloin is formed. For this reason, ketobutyrate was used as substrate to study the mechanism of these coupled dehydrogenase systems. The enzyme system has been fractionated and found to resemble the pyruvate dehydrogenase of *Escherichia coli* as described by Korkes *et al.* (*J. Biol. Chem.* 193: 721, 1951). The fractionated system is partially dependent upon the addition of lactic dehydrogenase in addition to 2 enzyme fractions of as yet unknown function, 1 of which separates at low saturation, the other at high saturation with ammonium sulfate. The appropriate acyl transferring system, (phospho-transpropionylase), which is present in the extracts in 80-fold excess, has not as yet been clearly separated from these fractions. In addition to the 4-protein fractions, the system is dependent upon coenzyme A, cocarboxylase, DPN and phosphate. Preparations from cells grown to contain the apodehydrogenase enzyme (O'KANE AND GUNSALUS *J. Bact.* 56: 499, 1948) require in addition lipoic acid—pyruvate oxidation factor—(REED, DEBUSK, GUNSALUS AND HORNBERGER *Science* 114: 93, 1951) for activation. The separated enzyme fractions have not as yet been freed of this activator.

Isolation of rat liver cell nuclei in gum arabic medium. ALEXANDER L. DOUNCE AND MORTIMER LITT.* *Dept. of Biochemistry, Univ. of Rochester, School of Medicine and Dentistry, Rochester, N.Y.*

The use of ice cold 1% gum arabic solution adjusted with NaOH to pH 6 instead of distilled water (DOUNCE, A. L. AND G. T. BEYER. *J. Biol. Chem.* 174: 859, 1948) is of very marked advantage in isolating cell nuclei from rat liver. Nuclei now can be obtained with only 4 or occasionally 3 centrifugations instead of 7 or 8. Dilute citric acid is used to adjust the pH of the homogenate to 6.0. The Waring Blendor now available is operated at 97 v. a.c. to homogenize the tissue (7–8 min. for 50 gm. frozen liver), and centrifugations are carried out in an International type II refrigerated centrifuge as follows: 2100 rpm, 30 min.; 1100 rpm, 15 min.; 800 rpm, 10 min.; and 600 rpm, 10 min. Using young rats (liver weight 7–10 gm.) there is little or no trouble with unbroken cells. Fiber is removed by straining the original homogenate through cheesecloth (curity grade 90) and then very fine cheesecloth (curity grade 120). Nuclei of rat liver prepared at pH 6.0 in 1% gum arabic have the same concentrations of aldolase, catalase, and desoxyribonucleic acid as nuclei prepared at pH 6.0 in distilled water. The microscopic appearance of the nuclei is not altered by the use of the gum. (Supported in part by the U.S.P.H. Service (N.C.I.) and the Fluid Research Fund, University of Rochester.)

Further studies of bacterial β-glucuronidase. MILES L. DOYLE* AND PHILIP A. KATZMAN. *Dept. of Biochemistry, Saint Louis Univ. School of Medicine, Saint Louis, Mo.*

The β-glucuronidase used in these studies was obtained by culturing *E. coli* at pH 6.8 in a completely synthetic medium composed of amino acids, sodium glycerolphosphate, menthyl glucuronide and inorganic salts. Attempts to purify the enzyme in these culture fluids by precipitation with ammonium sulfate or ethyl alcohol or by dialysis in each instance resulted in the separation of 2 relatively inactive fractions. When these fractions were combined, the resulting enzymic activity was markedly higher than the sum of the activities of the individual fractions. The evi-

dence indicates that enzymic activity is dependent on 2 components, the dialyzable and nonprecipitable fraction being relatively stable and extractable by butyl alcohol. These findings explain the previously reported loss of activity incurred by dialysis and ammonium sulfate precipitation of bacterial β-glucuronidase and present further evidence that this enzyme appears different from mammalian β-glucuronidase.

Unusual patterns of hemoglobin and stercobilin labeling with glycine-2-C^{14} in splenectomized dogs. DAVID L. DRABKIN, ASHTON GRAYBIEL* AND J. K. COLEHOUR.* *Dept. of Physiological Chemistry, Graduate School of Medicine, Univ. of Pennsylvania, Philadelphia, and U. S. Naval School of Aviation Medicine, Pensacola, Fla.*

Glycine-2-C^{14} was administered to 6 thoroughbred Beagle hounds of the same age, 3 of them splenectomized 8 months earlier, and the labeling of hemoglobin and stercobilin was followed during a period of 150 days. Both splenectomized and intact normal dogs respond to the anoxia of high altitude with an increase in red cells and hemoglobin. In the intact dog this is at least partly owing to the production of new cells. The maximum in hemoglobin and hemin labeling attained at 20 days and the early spike at 6–14 days with later maximum labeling at 90–100 days of stercobilin, usually observed in intact normal dogs after glycine-2-C^{14} administration, were not found in the splenectomized animals. The hemin labeling of the latter, both at sea level and high altitude, showed a gradual decrease from 3 days on, with no late increased labeling of the stercobilin. The splenectomized animals were not anemic, and the stercobilin data did not reflect any marked increase in blood destruction. These unusual patterns of hemoglobin and bile pigment labeling suggest the possibility of the existence of a hitherto unrecognized function of the spleen in the metabolism of red cells and hemoglobin.

Tri-o-cresyl phosphate as a vitamin E antagonist in the lamb and rat. HAROLD H. DRAPER,* M. F. JAMES* AND B. CONNOR JOHNSON. *Div. of Animal Nutrition, Univ. of Illinois, Urbana.*

Newborn lambs were fed a purified 'synthetic milk' diet deficient in vitamin E and containing tocopherol-low molecular-distilled lard as a source of fat. Those animals maintained on this basal diet for about 7 weeks exhibited either paralysis of the legs or anorexia and weight loss. Addition of tri-o-cresyl phosphate to the basal diet (0.4–1.0 gm/liter of 'milk' containing 19.5% dry matter) caused either severe leg weakness or sudden death after approximately 4 weeks. The effects of this compound could be delayed or prevented, but not cured, by oral administration of synthetic *dl*-alpha-tocopherol acetate at a rate of 100 mg/week. Lambs showing paresis induced by tri-o-cresyl phosphate ingestion died within 3 days despite the administration of large amounts of tocopherol either orally or intravenously as an emulsion. The outward manifestations of vitamin E deficiency obtained by feeding this diet, in addition to the muscular dystrophy, creatinuria and low plasma tocopherol levels also observed, indicate that the syndrome is identical with 'stiff-lamb disease' which occurs among the suckling offspring of ewes fed a vitamin E-low practical ration. Abnormalities in the electrocardiograms of paralytic lambs were also detected. In a corollary experiment, it was found that when the basal diet was fed to rats, the addition of tri-o-cresyl phosphate (2 mg/gm.) induced earlier development of a vitamin E deficiency, as identified by the hemolysis test of Rose and György, than was obtained on the basal regime alone. Supplementation with synthetic *dl*-alpha-tocopherol acetate (24 mg/100 gm. diet) prevented the occurrence of deficiency symptoms in rats receiving the above level of tri-o-cresyl phosphate in the diet over a 10-week period.

β-oxidation of fatty acids by a soluble enzyme system from mitochondria. GEORGE R. DRYSDALE (introduced by HENRY A. LARDY). *Inst. for Enzyme Research and Dept. of Biochemistry, Univ. of Wisconsin, Madison.*

Aqueous extracts of acetone powders prepared from rat liver mitochondria catalyze the β-oxidation of the straight chain even numbered fatty acids from butyric through lauric to yield acetoacetic acid. In a test system containing phosphate buffer at pH 7.4, Mg^{++}, caprylate and bovine plasma albumin, with ferricyanide or 2,6 dichlorophenol indophenol as electron acceptor, the system requires ATP or ADP for activity, adenylic acid being inactive. The enzyme remains in solution after centrifugation at 120,000 × *g* for 1 hour. The system is stable to freezing, to lyophilization, and to anaerobic dialysis, but it is inhibited by excessive amounts of dichlorophenol indophenol, ferricyanide, ATP or caprylate, and by arsenate in the presence of optimal amounts of the test system components. The preparation is unaffected by dinitrophenol at 10^{-4} M. Isotope experiments with carboxyl labeled butyric and caproic acids show that some randomization of the 2 carbon fragments occurs in acetoacetate formation from both of these fatty acids. When the protein solution is fractionated with acetone, one of the fractions retains slight activity; however, if it is combined with another of the fractions which has no activity alone, the maximal rate of oxidation is restored.

Role of B_{12} in methionine synthesis in *E. Coli* mutant 113-3. JACOB W. DUBNOFF. *California Inst. of Technology, Pasadena.*

Homocysteine, glutathione or cysteine partially replaces the B_{12} requirement of the *E. Coli* mutant 113-3. The growth promoting ability of these compounds is markedly enhanced by small amounts of B_{12}. This second requirement for B_{12} is at least partially replaced by the specific methyl donor, dimethyl-β-propiothetin provided homocysteine or its thiolactone is used to replace the major B_{12} requirement. Other methyl compounds such as choline, betaine, dimethylthetin, and dimethyl-γ-butyrothetin which are active in animals are completely inactive in this mutant.

Amphoteric properties of trypsin. J. A. DUKE AND M. BIER (introduced by F. F. NORD). *Dept. of Organic Chemistry and Enzymology, Fordham Univ., New York City.*

Measurements of the electrophoretic mobility of trypsin have shown that the isoelectric point of this enzyme is near pH 10.8. It appeared that a reasonable explanation of this unusually high value could be found in the amide-nitrogen content of the protein, equivalent to its content in aspartic and glutamic acid (M. BIER AND F. F. NORD. *Arch. Biochem. et Biophys.* 33: 320, 1951). For further study of the distribution of ionizable groups in trypsin, its electrometric titration curves were measured under a variety of conditions. The acid and base binding capacity was determined on commercial salt-free and on alcohol precipitated trypsin. At pH 2 the trypsin reaches a constant maximum acid binding capacity. The sigmoid curve between pH 2 and 6 indicates the ionization of 6 carboxyl groups for each 10,000 *g.* of protein. Calcium ions, in contrast to potassium, sodium, or magnesium ions, shift the dissociation curve to a lower pH. It is tempting to attribute this shift to a complex formation of calcium and the enzyme and to correlate it with the specific protective effect of calcium on the enzyme. In the alkaline region the titration curves were extended to the isoelectric point. However, due to the instability of the enzyme, there is some uncertainty in this region. The titration curves can be superimposed to plots of electrophoretic mobility versus pH. This is taken as evidence that acid and base binding capacity, as measured by electrometric titration, represents the net charge of the protein.

Synthesis of pyridine nucleotides in human erythrocytes in vitro. MARY DUNCAN,* HERBERT P. SARETT AND WILLIAM B. WENDEL. *Dept. of Biochemistry and Nutrition Research Lab., Dept. of Medicine, Tulane Univ. School of Medicine, New Orleans, La.*

The synthesis of pyridine nucleotide from nicotinic acid and nicotinamide in human erythrocytes *in vitro*, which was first observed by Kohn (*Biochem. J.* 32: 2075, 1938) and Leder and Handler (*J. Biol. Chem.* 189: 889, 1951), has been studied. Experiments were carried out variously on human defibrinated blood, on erythrocytes suspended in a physiological salt solution (Krebs-Ringers-phosphate), and on erythrocytes resuspended in serum. The cell suspensions were incubated at 37°C. in slowly rocking Erlenmeyer flasks through which a slow stream of moist gas was passed. Variations were made in gas composition, pH, substrate, and constituents of the medium with the following results. In experiments using concentrations of 0.1 mg.% nicotinic acid there was a 2-fold increase in pyridine nucleotide concentration during 24 hours of incubation. Synthesis was depressed by 95% O_2 but was not influenced by variations in O_2 tension ranging from 50%–0. Synthesis of pyridine nucleotide was not sensitive to pH changes (induced by changes in CO_2 tension) within physiological limits. High pH's (induced by 1% CO_2) measurably depressed synthesis. Added inorganic phosphate increased synthesis of pyridine nucleotide. When nicotinamide was used as substrate (20 mg/ml.) a 10-fold increase in pyridine nucleotide was noted. We have confirmed the observation of Leder and Handler that repeated washing of erythrocytes increases their ability to synthesize pyridine nucleotide. Incubation with gas mixtures (N_2–CO_2) containing only traces of O_2 had no influence upon synthesis of pyridine nucleotides from nicotinic acid, but decreased by about one-half synthesis from nicotinamide.

Automatic titrating and recording apparatus for microbiological assays. CHARLES H. EADES, JR.,* B. P. MCKAY AND W. E. ROMANS (introduced by T. P. NASH, JR.). *Dept. of Chemistry, Univ. of Tennessee, Memphis.*

An apparatus has been developed for automatically titrating and recording results in microbiological assays. It consists of an electronic controller, an automatic recording interval timer, an automatic sample changer, a plexiglass valve and a glass electrode-calomel electrode assembly. These units are coordinated through the electronic controller which is directed by the glass electrode pH indicator. Samples to be titrated are placed in a rack which will hold 144 culture tubes. The samples are automatically put in contact with the glass-calomel electrode assembly by a specially designed lifting device. A pH lower than 7 in the sample initiates titration. Alkali flows through a plexiglass valve operated by a solenoid; the time of flow through the calibrated delivery tube is registered by the interval timer

and recorded automatically when the pH has reached neutrality. The sample is then automatically changed according to a set program within the sample changer. The cycle is repeated until all 144 samples are titrated. This apparatus obviously can be used for any assay or measurement which involves acid-base titrations. Thus, it is adaptable to types of assays other than microbiological.

Metabolism of nitrogenous compounds by sunflower tissue cultures. F. S. Eberts, Jr. and A. J. Riker (introduced by R. H. Burris). *Depts. of Biochemistry and Plant Pathology, Univ. of Wisconsin, Madison.*

Sunflower tissue cultures in a medium containing a constant level of nitrate may show increased growth when very small amounts of alanine are added, but growth is inhibited by additional alanine and is negligible in the presence of 0.001 M alanine. Growth again increases as the concentration of alanine is raised from 0.004 M to 0.064 M, but is inhibited by more alanine than this. Glutamate and aspartate affect growth much as alanine does. To investigate this peculiar growth response, the nitrogen metabolism of tissue cultures was studied with the aid of compounds labeled with N^{15}. When $N^{15}O_3^-$ was supplied the cultures as the sole source of nitrogen for 4 hours, there was little difference in the N^{15} concentrations among the amino acids, with the exception of alanine, isolated from an acid hydrolysate of the tissue. The alanine contained a relatively high N^{15} concentration, but ammonia had the highest atom % N^{15} among the compounds isolated. Evidently ammonia is an intermediate in the reduction of nitrate to organic nitrogen by sunflower tissue cultures. When a mixture of $N^{14}O_3^-$ and N^{15}-labeled DL-alanine was furnished to tissue cultures as their source of nitrogen for 8 hours, a distinct differential in concentration of N^{15} was found among the amino acids isolated, although all were well labeled. Glutamic acid had the highest concentration of N^{15} exclusive of alanine, the compound supplied.

Effect of flavonoids on retention of ascorbic acid in rats and mice. Walter H. Eddy, Boris Sokoloff,* George Cone,* John Beaumont* and Rita Powella.* *Southern Bio-Research Lab., Florida Southern College, Lakeland.*

Sokoloff *et al.* revealed that the analogue of ascorbic acid, *d*-gluco-ascorbic acid, interferes with the synthesis of ascorbic acid but has only an insignificant effect on the utilization of dietary vitamin C. 100 Ak leukemic mice were divided into two groups of 50 animals each. One group was placed on a Sherman-LaMer scorbutogenic diet to which 5% of *d*-gluco-ascorbic acid and 0.5% of citrus flavonoids were added. Five days later, *d*-gluco-ascorbic acid was reduced to 3%. The average level of ascorbic acid for the treated mice was 0.04–0.05 mg/100 cc. of plasma. The number of white blood cells gradually reached a subnormal level of 2500. All but 3 mice survived, remaining in good condition for several weeks. The second group was placed on the same regime without flavonoids. The ascorbic acid level went down close to zero with an average of 0.01 mg/100 cc. of plasma. The mortality rate in this group was 65%, the rest remaining in poor health and manifesting a tendency to bleeding. Similar observations were made on normal rats and mice. The ascorbic acid content of the adrenal was higher in the first group. These observations seem to indicate that citrus flavonoid compound increases the retention of ascorbic acid in blood and the adrenal glands as far as rats and mice are concerned.

Blood nonprotein-nitrogen constituents during barbiturate addiction and withdrawal. Anna J. Eisenman, Jewell W. Sloan* and H. F. Fraser.* *NIMH Addiction Research Center, USPHS Hosp., Lexington, Ky.*

Fifteen human subjects were studied. During withdrawal of barbiturates there is usually a transient (1–23 mg. %) rise of blood total nonprotein-nitrogen which may occur anytime from the 3rd to the 8th day of withdrawal. Following convulsive seizures during withdrawal, the serum uric acid shows striking rises, correlated with eosinopenia, sometimes increasing by 10 mg. %. The maximum increase in serum uric acid is reached 2–5 hours after a convulsion. Unless the level is reinforced by a 2nd or 3rd convulsion, it decreases sharply at first, then more gradually to below control (addiction) values. The increase in uric acid usually precedes that in NPN and is not sufficient to account for the increment in the total NPN. The relation between whole blood and serum NPN and urea seems to be consistent throughout addiction and withdrawal. Creatine and creatinine (serum) changes were not significant. Some of the NPN increase is due to urea, the remainder of the increase is not accounted for by the constituents we have studied. The elevated uric acid values after withdrawal convulsions could be due to convulsions alone, unassociated with barbiturate withdrawal. Four psychiatric patients receiving electroshock for the first time were used as controls. These subjects received a series of 3 shocks at 2- or 3-hour intervals. Uric acid rose about 6 mg. after the second shock. The increase was further reinforced by the third shock of the series. The rate of falling off was variable.

Effect of ascorbic acid pretreatment on adrenal response to stress. ALBERT B. EISENSTEIN AND JOAN BONIFACE (introduced by ETHEL RONZONI). *Depts. of Preventive Medicine and Neuropsychiatry, Washington Univ. School of Medicine, St. Louis, Mo.*

The adrenal cortex contains large quantities of cholesterol and ascorbic acid. Although isotopic studies have shown cholesterol to be a direct precursor of adrenocortical hormones, the role of ascorbic acid in adrenal function is not completely understood. It has been suggested by Dugal and Therien that ascorbic acid may play a role similar to adrenal hormones because of its ability to prevent stress hypertrophy. The purpose of the present investigation was to elucidate the role of ascorbic acid in the adrenal response to stress. Two groups of male rats were stressed with a single dose of epinephrine. Adrenal cholesterol and ascorbic acid determinations were done prior to, 3, and 24 hours after stress. Control animals at 3 hours showed marked reduction in ascorbic acid and cholesterol and at 24 hours both constituents had returned to resting levels. Animals pretreated with large doses of ascorbic acid demonstrated a significant elevation of the resting cholesterol level but their response to stress was almost identical to that of the controls. Other groups of animals were subjected to stress by cold, 3° for 3 days. Adrenal hypertrophy occurred in controls but not in those receiving vitamin C. The adrenal cholesterol concentration was greater following this stress in animals receiving ascorbic acid. Although recent investigations indicate that ascorbic acid is not directly concerned with elaboration of adrenal cortical hormones, these observations suggest that it may indirectly influence their production by increasing the adrenal cholesterol content. (Supported in part by a grant from the Nutrition Foundation, Inc.)

Purine metabolism of diaminopurine-resistant *Lactobacillus casei*. GERTRUDE B. ELION AND M. EARL BALIS (introduced by GEORGE B. BROWN). *Wellcome Research Labs., Tuckahoe, N. Y., and the Labs. of the Sloan-Kettering Inst. for Cancer Research, New York City.*

The purine metabolism pattern of a 2,6-diaminopurine-resistant mutant of *Lactobacillus casei* (obtained through the courtesy of Dr. B. Davis) has been investigated. The mutant differs noticeably from the wild strain in a number of respects. Its ability to use adenine for growth, in a thymine-containing medium, is greatly diminished, supporting the view expressed earlier that the inhibitory effects of diaminopurine are directed toward specific adenine-utilizing mechanisms. A number of nucleosides and nucleotides support growth of both strains, but the mutant can grow with crotonoside (isoguanine riboside) or 2,6-diaminopurine riboside as the sole purine source, while the wild strain cannot. Differences between the strains have also been noted with respect to their responses to certain unnatural purines and purine analogs. Adenylic acid *b* is greatly superior to the *a* isomer as a precursor of the nucleic acids in both strains.

Utilization of D-amino acids by *Tetrahymena geleii*. ALFRED M. ELLIOTT, JAMES F. HOGG AND CHUNG WU (introduced by LAWRENCE H. LOUIS). *Depts. of Zoology and Biological Chemistry, Univ. of Michigan, Ann Arbor.*

Tetrahymena geleii E requires for growth the amino acids serine, threonine, valine, leucine, isoleucine, lysine, arginine, histidine, methionine, tryptophan and phenylalanine. The aim of this investigation was to determine the utilization of the D-isomers of these 11 amino acids by this organism. The D-isomers were tested in synthetic media which were deficient in the appropriate amino acids. The basal media contained no D-amino acids. Each D-amino acid was tested at zero and suboptimal levels of the corresponding L-amino acid. For control tests, media were prepared which contained these same levels of the L-amino acid without the D-isomer. The D-amino acid was tested in each of the above media at 3 levels; one equalled the suboptimal level of the L-form and the other two were 50% and 100% of the level of the L-amino acid in the complete basal medium. The culture tubes were inoculated aseptically with washed suspensions of cells from either a peptone or a synthetic medium culture of *T. geleii*. While the series without L-amino acid determines a replacement by the D-amino acid, the other series elucidates a sparing or inhibitory action of the D-amino acid. Growth was measured turbidimetrically every 24 hours after inoculation until maximal growth had developed in the complete basal medium. The results showed that only D-methionine, D-lysine and D-arginine were active for this organism; D-leucine was inhibitory. The other D-amino acids were completely inactive. The results were qualitatively identical for each type of culture employed. These results are to be compared with those of resting cell studies.

Enzymatic synthesis of glutamine. WILLIAM H. ELLIOTT (introduced by FRITZ LIPMANN). *Biochemical Research Lab., Massachusetts General Hosp., Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.*

The isolation of the enzyme system which synthesizes glutamine from ATP, glutamic acid and ammonia has been undertaken with a view to

elucidating the mechanism of the reaction. Extracts of green-pea meal have been used as a convenient starting material; fractionation with protamine and nucleic acid combined with ammonium sulfate precipitation has yielded highly active preparations with over a thousand-fold purification. There has been no indication that more than one protein is involved in the synthesis, nor has there been an indication for the participation of a coenzyme. In addition to the formation of a hydroxamic acid from glutamic acid, the enzyme preparation shows an intense 'glutamyltransferase' activity. This formation of a hydroxamic acid from glutamine requires trace amounts of ATP or ADP and phosphate as described by Stumpf *et al.* (*Arch. Biochem.* 30: 126, 1951) for the transferase of pumpkin seedlings; other incubation conditions were the same as those used for glutamine synthesis. The enzyme concentrate is now being used for further exploration of the mechanism of the glutamine formation.

Aspects of mechanism of formation of methyl groups from serine. David Elwyn (introduced by David B. Sprinson). *Dept. of Biochemistry, Columbia Univ., New York City.*

Investigations of the synthesis of the methyl groups of choline and thymine from 2,3-deuterio-3-C^{14}-L-serine have indicated that formate or its derivatives are not involved as intermediates since C-H bonds are apparently unbroken (Elwyn, Weissbach and Sprinson, *J. Am. Chem. Soc.* 73: 5509, 1951). The preparation of the serine by reduction of ethylformylhippurate with Al-Hg and D_2O may have resulted in the attachment of deuterium in only one of the two possible positions on the β-carbon atom of L-serine. The equal dilution of C^{14} and D in the synthesis of methyl groups might have been due to participation of the α-deuterium or to selective enzymatic cleavage of C–H bonds rather than C–D bonds. In either case formate would not be definitely excluded as an intermediate. L-Serine was therefore prepared by reduction of ethyl (D, C^{14}-formyl)hippurate with Al–Hg and normal water. This results in labeling only in the β position and, if an asymmetric synthesis was achieved, the deuterium is attached in a configuration opposite to that of the first serine. Administration of this amino acid to rats also resulted in equal dilution of C^{14} and D in the synthesis of the methyl groups of choline, in agreement with the previous findings. The synthesis of methyl groups from other precursors will also be discussed.

Enzymatic phosphorylation of riboflavin to flavin mononucleotide in animal tissues. Sasha Englard (introduced by Merton F. Utter). *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

A preparation has been obtained from small intestines of rat which catalyzes the reaction: riboflavin + ATP → riboflavin-5-PO_4 + ADP. Previous reports (*Naturwiss* 23: 286, 1935; *Enzymologia* 6: 333, 1939) that extensive phosphorylation of riboflavin occurred in the presence of inorganic phosphate by the reversal of intestinal phosphatase action could not be repeated. In the absence of ATP no FMN was synthesized in the present studies. No FAD could be detected at any time over an incubation period up to 2 hours from either FMN or riboflavin. This indicates the probable absence from the preparation of the enzyme described by Schrecker and Kornberg (*J. Biol. Chem.* 182: 795, 1950) which condenses FMN and ATP to yield FAD and pyrophosphate. This also suggests that the FMN is formed as a direct product of the phosphorylation of riboflavin rather than by a prior synthesis of FAD by an unknown mechanism and its subsequent breakdown to FMN. Zn^{++} has been found to increase the synthesis by two fold but this stimulation has been found to be due to the inhibition of the phosphatase activity by this cation. These results, plus the fact that aging seems to differentially inhibit the phosphatase activity indicates that the phosphorylation of riboflavin by intestinal extracts is not due to the reversible action of a non-specific phosphatase. The enzymatically synthesized riboflavin-5-PO_4 has been identified as flavin mononucleotide by its distribution coefficient between benzyl alcohol and water and by its ability to activate the apoenzyme of cytochrome *c* reductase of yeast.

Stability of various penicillin salts used for growth promotion in animal feeds. Raymond G. Esposito* and William L. Williams. *Lederle Labs. Division, American Cyanamid Co., Pearl River, N. Y.*

The turbidimetric method (*J. Biol. Chem.* 153: 249, 1944) for determination of penicillin activity is unreliable for assay of penicillin in animal feeds due to the presence of non-antibiotic materials, inhibitory to the assay organism. A new method has been devised based on that of Vincent and Vincent (*Proc. Soc. Exper. Biol. & Med.* 55: 162, 1944). Sample extracts are pipetted on filter paper discs which are placed on a solid inoculated medium. Zones of inhibition are measured after 6 hours' incubation. The particular advantage of this method is that interference due to non-antibiotic inhibitory materials is eliminated. Using a methanol extract of penicillin supplemented feed, reliable assays can be obtained with a concentration of 1 unit penicillin activity per milliliter of extract. This new specific penicillin method has been applied in a study of the stability of penicillin salts in feeds during storage and

pelleting. Various penicillin salts were added to a feed at the rate of 25 gm/ton. The feed was pelleted in a commercial mill. Procaine penicillin showed an average destruction of 48%; di-para-chlorophenylbiguanide penicillin, 90%; and paludrine penicillin, 88%. During a brief steam treatment designed to simulate the pelleting process the percent destruction of various penicillin salts were: procaine penicillin, 50%; 'diamine penicillin,' 72%; and di-parachlorophenylbiguanide penicillin, 55%. In unpelleted feeds after 2 months storage, average destruction of procaine penicillin was 4%; di-parachlorophenylbiguanide penicillin, 58% and paludrine penicillin, 71%.

Host nitrogen as a source for T_7 bacteriophage. E. A. Evans, Jr., Frank W. Putnam and Lucille Palm.* *Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.*

In contrast to T_6 bacteriophage, T_7 derives most of its P from the host bacterium, *Escherichia coli* (*Federation Proc.* 10: 181, 1951). The present experiments demonstrate that most of the N, as well as the P, of the *nucleic acid* of T_7 originates in high molecular weight compounds synthesized prior to infection, probably desoxyribonucleic acid (DNA). However, more than half the N of the phage *protein* is incorporated from NH_4Cl of the medium. Bacteria were labeled by growth in synthetic medium containing lactate, $N^{15}H_4Cl$ and $Na_2HP^{32}O_4$ and were infected with up to 10 T_7 particles per cell. Lysis and phage release began at 15 to 20 minutes increasing to a maximum at 50 minutes, as indicated by viable cell count, turbidity, and plaque count. However, total DNA and RNA remained essentially constant. Phage progeny, isolated by centrifugation and characterized by infectivity and sedimentation velocity measurements, were partitioned chemically and analyzed for isotopic activity. Both for N^{15} and P^{32} the isotopic activity of virus nucleic acid was 65 to 95% that of the original cell and generally exceeded that of bacterial acid soluble N or P. The atom percentage excess N^{15} of phage protein was only half that of phage DNA. The latter finding accords with results for T_6 indicating the existence of different host precursors of virus nucleic acid and protein. The greater utilization of host substance for synthesis of T_7 than T_6 apparently results from smaller size and requirements of T_7 rather than from fundamental differences in reproduction.

Reversibility of mercuric chloride inhibition of urease. H. E. Evert (introduced by C. L. Gemmill). *Dept. of Pharmacology, Univ. of Virginia Med. School, Charlottesville.*

The observations obtained by the colorimetric SH determination concerning the SH group variation during urease activity previously reported (*Federation Proc.* 10, 246, 1950) have been extended by the inhibition-reactivation method. A series of mercuric chloride inhibition curves were obtained by using varied urea concentrations from 0.83–2.5 $\times 10^{-6}$ moles/10 ml. in the reaction. The urea decomposed in 10 minutes was plotted against the mercuric chloride concentration. With the use of these curves, the concentration of mercuric chloride required for 50% inhibition was plotted as a function of the ureolytic activity. Parabolic or bell-shaped curves were obtained that were similar to those obtained by the colorimetric SH analysis. In addition, it was determined that the concentration of thiol compound required for urease reactivation after mercuric chloride inhibition of urease varied in such a manner to support the above observations. For example, a higher concentration of thiol compound was required for reactivation at 2.1 $\times 10^{-6}$ moles urea/10 ml. than at 2.3 $\times 10^{-6}$ urea/10 ml. The mercuric chloride inhibition, therefore, is dependent upon the free SH variation and upon the urea concentration in the reaction. The SH variation during urease activity appears to be related to the formation of a urea-urease complex as was suggested by the results obtained by the colorimetric SH method.

Photochemical carbon dioxide fixation by cell-free leaf macerates. E. W. Fager (introduced by H. Gaffron). *Inst. of Radiobiology and Biophysics (Fels Fund) Univ. of Chicago, Chicago, Ill.*

Cell-free leaf macerates which show a small but definite and reproducible increase in carbon dioxide fixation upon illumination have been prepared by using low temperatures and anaerobic conditions. Over 75% of the carbon dioxide fixed by such preparations in both the light and the dark is found in phosphoglyceric and pyruvic acids; over 70% of the increase due to illumination is found in these compounds. Illumination increases the amount fixed in the neutral fraction (presumably sugars) by a factor of 4. The ratio, increase in fixation due to light/fixation in dark, averages 0.8. Addition of various 2-carbon compounds more than doubles this value. Fractions obtained from the proteinaceous material remaining in solution after removal of chloroplast material by centrifugation give, when recombined with the chloroplast material, values of 5 or more for this ratio.

Inactivation of lysozyme by trace elements. R. E. Feeney, E. D. Ducay and L. R. MacDonnell (introduced by Hans Lineweaver). *Western Regional Research Lab., U. S. Dept. of Agriculture, Albany, Calif.*

Lysozyme, an unusually stable biologically active protein, is rapidly inactivated by small amounts of Cu. Lysozyme solutions stored without

added metal ions at room temperature and at pH 4.0 retained 77% of original bacteriolytic activity after 6 years. At pH 8.0 and 9.0, activities of 10 and 3% were retained. Cu (1–10 ppm.) greatly increases loss in alkaline solution. At pH 9.3 and 47°C., 0.05% lysozyme solutions in borate or phosphate buffers lost 50% activity in 90 minutes with 10 ppm. Cu, but lost no activity even in 48 hours without added Cu. Ag, Hg and Pd caused apparent activity decreases but these metals precipitated the protein which made interpretations difficult. Ni gave slight effects. Fe, Co, Zn, Mn, Cr, Sn and Ca have little or no effect. Complexing agents (NH_3, glycine, and ethylenediamine tetracetic acid) prevented the Cu reaction. The Cu reaction appeared to be independent of O_2 and light, and to have a Q_{10} of 5–6 for the temperature range 27°–47°C. Inactivation was not accompanied by losses in amide N or in any of 13 amino acids tested, except for tyrosine, which showed definite, but perhaps unrelated, decreases. Cystine may be involved in the reaction, but reliable data are not available because cystine analyses were erratic in presence of Cu. Preliminary results with ovomucoid (trypsin inhibitor) indicate similar effects by Cu.

Inhibition of xanthine oxidase by ascorbic acid. PHILIP FEIGELSON (introduced by P. ROTHEMUND). *Div. of Biochemistry, Fels Research Inst., Antioch College, Yellow Springs, Ohio.*

In the course of an investigation on the nature of the unknown prosthetic group of xanthine oxidase, it was observed that incubation of a purified cream xanthine oxidase preparation with trace amounts of ascorbic acid resulted in a marked loss of xanthine oxidase activity. The extent of inhibition appears to be a function of the concentration of ascorbic acid as well as the length of time that the enzyme and ascorbate are incubated together in the absence of substrate. Following the procedure of Ackermann and Potter (*Proc. Soc. Exper. Biol. & Med.* 72: 1, 1949) measurements of the enzyme activity in the absence and presence of ascorbate at various enzyme levels indicate that the ascorbate may act an an irreversible inhibitor. The mechanism and significance of these findings are discussed.

Influence of cysteine and thyroxine on oxidative phosphorylation. GLADYS FELDOTT* AND HENRY A. LARDY. *Inst. for Enzyme Research and Dept. of Biochemistry, Univ. of Wisconsin, Madison.*

Thyroxine in concentrations of 10^{-5}–10^{-6} M inhibits the oxidation of glutamate and accelerates the oxidation of fatty acids by cell free preparations from rat kidney (*Federation Proc.* 10: 182, 1951). While studying the mechanism of this *in vitro* effect of the hormone, a relationship was found between thyroxine and cysteine. Cysteine, at concentrations of 10^{-4}–10^{-3} M, produced a profound initial stimulation of glutamate oxidation by rat liver mitochondria preparations, followed by an inhibition. Thyroxine, at 10^{-5} M, completely prevented both the initial stimulating effect of cysteine and the inhibition which followed. In the absence of added cytochrome *c*, cysteine was without effect on the oxidation of glutamate. Cysteine depressed the P/O ratio from 3–2 in a mitochondrial system oxidizing glutamate. However, when the glutamate oxidation was blocked at the succinate stage with malonate, cysteine almost completely uncoupled phosphorylation from oxidation. Thyroxine overcame the uncoupling effect of cysteine in either the presence or absence of malonate. Under these conditions, thyroxine alone had no effect on either oxidation or phosphorylation. The uncoupling effect of cysteine is an adequate explanation of its stimulation of glutamate oxidation.

Consumption of thromboplastin in blood coagulation process. SILVIO FIALA, D. R. MERANZE, KARL ROTH (introduced by CECILIA RIEGEL). *Dept. of Research, Albert Einstein Med. Center, Mount Sinai Hosp., Philadelphia, Pa.*

The incubation at 38°, in the presence of Ca ions, of defibrinized human plasma with diluted tissue thromboplastin (brain or placenta), shows thromboplastin consumption detectable after thrombin has been neutralized by antithrombin and the effect of the formed stable prothrombin conversion factor removed by heating. Also serum under analogous conditions inactivates thromboplastin, the amount of inactivated thromboplastin being dependent on the concentration of serum. This reaction, prevented by heating of serum to 56° and lessened by adsorption on kaoline, is different from complement fixation. The factor responsible for inactivation of thromboplastin has adsorption affinities similar to prothrombin but exists also in prothrombin free serum and probably is identical with serum prothrombin conversion factor. While the thromboplastic system of blood is not strictly identical with tissue thromboplastin an analogous consumption of prothrombin conversion factors and of antihemophilic globulin occurs also. No thrombokinase potency in serum is detectable. The meaning of these findings for the theory of blood coagulation will be discussed.

Significance of increased alveolar-arterial oxygen tension difference during exercise. GILES F. FILLEY, FERNAND GREGOIRE AND GEORGE W. WRIGHT (introduced by F. W. KLEMPERER). *Dept. of Physiology, E. L. Trudeau Fndn., Saranac Lake, N. Y.*

Alveolar and arterial oxygen tensions were determined in 36 normal men aged 23–60 breath-

ing air at rest and during exercise requiring an average oxygen consumption of 1.08 l/min/m.^{2} The alveolar pO_2 was calculated from the alveolar equation substituting arterial pCO_2 for alveolar pCO_2. Arterial gas tensions were determined by a modification of the Riley bubble method. The mean difference between alveolar and arterial pO_2 was 9.7 ± 5.27 mm.Hg at rest and 19.6 ± 4.74 mm.Hg during exercise. Of the 4 commonly given explanations for an Aa difference at rest or during exercise, namely *1*) failure of pulmonary capillary blood to reach alveolar oxygen tension ('membrane factor'), *2*) the admixture of venous or partially oxygenated blood with pulmonary capillary blood ('venous admixture'), *3*) a change in the physico-chemical state of the blood in transit from lung to artery, *4*) analytical errors, the membrane factor or venous admixture appear to be the most likely. On the assumptions that there is no membrane factor at rest and that the same percentage of venous admixture occurs during exercise as occurs during rest, calculations from our data predict that the Aa difference during exercise should be increased by approximately the values that we actually observed in this study. This constitutes evidence that it is not necessary to postulate a membrane factor in order to explain the increase of Aa difference that occurs during exercise. (Supported by Contract N7onr-307, Task Order 3 NR 112-214 with the Office of Naval Research.)

Effect of partial hepatectomy, protein realimentation, bile, 1% methionine diet, ether and chloroform on biliary secretion in the rat. BERNARD FISHER,* JOAN W. ZERBE* AND HARRY M. VARS. *Harrison Dept. of Surgical Research, Univ. of Pennsylvania Schools of Medicine, Philadelphia, Pa.*

Using the method of collecting rat bile described by us (*Am. J. M. Sc.* 222: 151, 1951), the volume, total cholic acid and total cholesterol secretion were studied in adult male Wistar rats subjected to 70% partial hepatectomy. By the 4th postoperative day, cholic acid and cholesterol output was equal to that of control animals, while bile volume did not return to control levels in 14 days. Three week protein-depleted rats transferred to an 18% protein diet 4 days after operation secreted cholic acid equivalent to normal protein-fed animals in 24–48 hours. Volume did not return to normal for 4–5 days, while total cholesterol remained low during 8 days of protein feeding. Rat bile fed to biliary fistula animals increased cholic acid output to 80–90% of expected values with no change in cholesterol output, and a 10 to 15% increase in bile volume. Protein-depleted animals fed a 1% methionine diet for 7 days increased total cholic acid output by 25–50%, with a slight increase in bile volume but not of cholesterol. Deep ether anesthesia (10 and 20 min.) produced no effect or a slight increase in cholic acid secretion and bile volume. Bile cholesterol was unaffected. Recovery from the depressant effects of chloroform on bile composition was rapid. (This work was done under contract number DA-49-007-MD-143 between the University of Pennsylvania and the Department of the Army.)

Direct enzymic transfer of hydrogen atoms between substrates and DPN. HARVEY F. FISHER,* P. OFNER,* ERIC E. CONN,* BIRGIT VENNESLAND AND F. H. WESTHEIMER.* *Depts. of Biochemistry and Chemistry, Univ. of Chicago, Chicago, Ill.*

We have previously reported (*J. Am. Chem. Soc.* 73: 2403, 1951) that alcohol dehydrogenase catalyzes a direct transfer of deuterium from the α-carbon of CH_3CD_2OH to DPN. The reduced DPN (deutero DPN or DPND) so formed has now been reoxidized enzymically with acetaldehyde. The oxidized DPN contained no appreciable deuterium. On the other hand, chemically reduced DPND (reduced with hydrosulfite in D_2O) lost about one half of its deuterium on enzymic reoxidation. These experiments show that only one of the two possible diastereomers is formed when the pyridine ring is reduced enzymically with deutero ethanol; or, in other words, the deuterium is transferred only to one side of the plane of the pyridine ring. DPND was also reoxidized enzymically with pyruvate in the presence of lactic dehydrogenase. The lactate formed contained one atom of deuterium per molecule when enzymically reduced DPND was employed, but only approximately one half an atom of deuterium was found per molecule of lactate when chemically reduced DPND was employed. These experiments show that lactic dehydrogenase catalyzes a direct, stereochemically specific, transfer of hydrogen atoms from pyridine nucleotide to substrate. Furthermore, this enzyme catalyzes the transfer of hydrogen to and from the same side of the pyridine ring as does alcohol dehydrogenase. The fact that hydrogen atoms are transferred directly shows that the enzymic oxido reduction cannot involve the formation of an intermediate which exchanges hydrogen with the medium. Transfer of hydrogen through a sulfhydryl group thus is improbable.

Relation of dietary protein to signs of choline deficiency—effect of supplements of vitamin B_{12} and folic acid. MARIE A. FISCHER AND GLADYS D. HALL (introduced by WILLIAM S. MCELLROY). *Dept. of Physiological Chemistry, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.*

By the tenth day 75% of the rats fed a diet con-

taining 15% casein showed signs of acute choline deficiency. The kidneys were enlarged and hemorrhagic, the serum non-protein nitrogen had risen to 70 mg. % and the livers contained an excess quantity of fat. Addition of B_{12} and folic acid to this diet reduced liver fat and prevented kidney damage. Both control and supplemented groups fed a 9% casein ration exhibited poor growth and neither group showed signs of choline deficiency, however, the lipotropic effect of the supplement was evident. When animals were fed a 24% casein diet the vitamin supplements had no apparent effect on growth response, liver weight and fat content, or kidney weight. The serum NPN of both of these groups was about 30 mg.%. In contrast, the supplement reduced the NPN from 26 mg.% to 20 mg.% when the animals were maintained on 9% casein. Low values were also observed when the supplemented 15% casein diet was fed. Serum albumin content was low in animals fed the 9% casein diet. B_{12} and folic acid additions to the diet slightly raised the level. The supplements had least effect on serum albumin of rats fed the 24% casein diet. Changing the protein content or adding the vitamins to the diets caused some changes in the serum globulins.

Enzymatic phosphorolysis of maltose with production of β-D-glucose-1-phosphate. CHARLOTTE FITTING* AND MICHAEL DOUDOROFF. *Dept. of Bacteriology, Univ. of California, Berkeley.*

In previous studies by Fitting and Scherp (*J. Bact.*, in press), it had been shown that the utilization of maltose, but not of glucose, by *Neisseria meningitidis* (strain 69, type I) results in the accumulation of a labile phosphate ester. Enzyme preparations of this organism were found to catalyze a reversible phosphorolytic decomposition of maltose with the liberation of equal amounts of β-D-glucose-1-phosphate and glucose. The ester was isolated by barium fractionation. It was found to be non-reducing and completely hydrolyzable in *N* acid within 7 minutes at 100°C. It was compared to chemically synthesized β-D-glucose-1-phosphate; the two compounds were identical with respect to optical rotation and phosphorus and glucose content. Both the isolated and the synthetic β-D-glucose-1-phosphate could serve as substrates for the enzyme which in the presence of glucose produces inorganic phosphate and a reducing sugar. This reducing sugar is not attacked by *Torula monosa* but it is completely fermented by *Saccharomyces cervisiae* and it has the same Rf value as maltose when developed with a mixture of butanol, ethanol and water. *Alpha*-glucose-1-phosphate, which is produced in the phosphorolysis of starch, glycogen and sucrose, could not be detected as a product of maltose breakdown nor did it serve as substrate for this enzyme. (This work was supported in part by a USPHS Fellowship from the National Microbiological Institute.)

Metabolism of octamethyl pyrophosphoramide (OMPA) by liver slices and homogenates. JOSEPH H. FLEISHER* AND BERNARD J. JANDORF. *Enzyme Chemistry Branch, Chemical Corps Med. Labs., Army Chemical Center, Md.*

The *in vitro* conversion of the weak anticholinesterase OMPA into a more powerful one by liver slices (DUBOIS *et al.*, *J. Pharmacol. & Exper. Therap.* 99: 376, 1950) has been confirmed. Thus, a standard preparation of plasma esterase was inactivated to a negligible extent by 2×10^{-3} M OMPA while the same enzyme preparation was 60% inactivated after preliminary incubation of OMPA in the presence of rat liver slices. Since some loss of the intrinsic anticholinesterase activity of OMPA occurs during incubation without tissue, the true increase in potency is probably even higher than the measured one. The weak anticholinesterase activity of OMPA is destroyed within a few minutes upon incubation with 5% rat liver *homogenate*. On the other hand, the activity produced by the action of liver slices on OMPA is decreased to only a minor degree upon subsequent incubation with liver homogenate (20 hours, 25°). The mechanism of these activations and inactivations is being studied further; preliminary manometric experiments indicate that the inactivating action of homogenates on OMPA is not accompanied by stoichiometric acid formation as is the case with tetraethyl pyrophosphate.

Changes in free amino acids of brain after hepatectomy. EUNICE V. FLOCK, MELVIN A. BLOCK,* FRANK C. MANN,* JOHN H. GRINDLAY* AND JESSE L. BOLLMAN.* *Mayo Fndn., Rochester, Minn.*

A large increase in concentration of free amino acids of the brain occurs following total removal of the liver in the dog. This increase is progressive and does not appear to be affected by the amount of glucose administered to the dehepatized dog; in contrast, the increase of amino acids in the plasma was greater when no or minimal amounts of glucose was given than when large amounts were infused. The individual free amino acids have been separated by paper chromatography and glutamine has been observed to be much more abundant in the brain after hepatectomy than in the normal brain. Quantitative determinations of total α-amino nitrogen and of glutamine have been made by the methods of Hamilton and Van Slyke. In the cerebrums of 5 normal dogs the mean concentration of glutamine was 4.6

± 0.70 mg. α-amino N/100 gm. and the total α-amino nitrogen was 24.1 ± 2.0 mg. In the cerebrums of 10 dehepatized dogs surviving from 13.2–24.2 hours after operation the mean concentration of glutamine was 27.4 ± 1.7 mg. and the total α-amino nitrogen was 54.0 ± 2.4. Similar values were found for the cerebellum. The 6-fold increase in glutamine would account for approximately 75% of the total increase in free amino acids. Glutamic acid in brain did not decrease greatly after hepatectomy. Glutamine also increased after hepatectomy in skeletal muscle but here this was associated with a marked decrease in glutamic acid and little change in other amino acids.

Fate of procaine and 2-chloroprocaine in the subarachnoid space. FRANCIS F. FOLDES, ROBERT D. HUNT AND DAVID L. DAVIS (introduced by L. REINER), *Dept. of Anesthesia, Mercy Hosp. and Section on Anesthesiology, Dept. of Surgery, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.*

A thin vinylite catheter was introduced through a #18 spinal needle into the subarachnoid space of patients who were to receive spinal anesthesia. Following the removal of the needle, the patients were placed in the supine position and 100 mg. of procaine or 2-chloroprocaine with or without 0.6 mg. of epinephrine was administered through the vinylite catheter to 10 patients each. The volume of the injection was made up to 3.0 ml. with 10% dextrose. The level of sensory anesthesia was allowed to progress to the 10th dorsal dermatome. Two-tenths ml. spinal fluid samples were taken immediately, at 1, 3, 18, 33, 48, 63 and 78 minutes after the subarachnoid injections. The procaine or 2-chloroprocaine concentration of the samples were determined by Ting method (*J. Lab. & Clin. Med.* 34: 822, 1949). The concentration of procaine and 2-chloroprocaine decreased at first rapidly, then more slowly in all experiments. However, there was a marked variation between the concentrations found in corresponding samples taken from different patients. The concentration of 2-chloroprocaine in the last sample was somewhat lower than that of procaine, and in general the final concentrations of both procaine and 2-chloroprocaine were higher if they were injected with epinephrine. The initial rapid decrease of the concentration of procaine or 2-chloroprocaine could not be wholly explained by diffusion in the spinal fluid or by vascular absorption. To a large extent absorption by the spinal cord and the nerve roots must account for the disappearance of these drugs from the spinal fluid.

Effect of alcohol intoxication on the cholesterol and ascorbic acid content of guinea pig adrenal glands. J. C. FORBES AND G. M. DUNCAN.* *Dept. of Biochemistry, Med. College of Virginia, Richmond.*

The administration by stomach tube of 5–7 gm. of ethyl alcohol/kg. of body weight to half-grown male guinea pigs caused severe intoxication followed by a marked reduction in both the cholesterol and ascorbic acid content of the adrenal glands. This effect came on slowly, no demonstrable change being evident in 7 hours but by the end of 24 hours the concentration of each constituent had dropped to around one half its original value. The ascorbic acid level showed very little tendency to rise following the initial drop unless ascorbic acid was administered, in which case the daily intraperitoneal injection of 50 mg. sodium ascorbate raised the adrenal level to normal in 96 hours. The cholesterol content tended to rise slowly and was close to normal in a few animals 96 hours after the alcohol administration, but in the majority the level remained low. The rate at which it returned to normal was slightly accelerated by the daily injection of sodium ascorbate. Studies on the effect of ascorbic acid intake on the LD_{50} of alcohol for guinea pigs also will be presented. (We gratefully acknowledge a Grant-in-Aid from the Committee on Problems of Alcohol, National Research Council, in support of this investigation.)

Enzymatic fractionation of adenosine pyrophosphates in blood. PETER FORIS,* BRUCE MACKLER* AND GEORGE M. GUEST. *Children's Hosp. Research Fndn., Dept. of Pediatrics, Univ. of Cincinnati, Cincinnati, Ohio.*

Potato apyrase (*Arch. Biochem.* 20: 261, 272, 1949) and yeast hexokinase (*J. Gen. Physiol.* 29: 379, 1946) have been used for determinations of adenosinetriphosphate (ATP) and adenosinediphosphate (ADP) in whole blood. *A*) Potato apyrase incubated with deproteinized blood filtrates (perchloric acid pptn) liberates labile P from both ATP and ADP. *B*) Incubation of the blood filtrate first with hexokinase and glucose (ATP + glucose → ADP + glucose-6-PO_4), then with apyrase, yields the total apyrase labile P minus one half the labile ATP phosphorus. The difference between the two determinations (*A-B*) of hydrolyzed P represents one half the labile P of ATP. The ADP phosphorus is then calculated by deducting the labile P derived from ATP from the total apyrase labile P. Results from parallel determinations of enzyme-hydrolyzable P by this method and of acid-hydrolyzable P by the Lohmann method will be reported. (Aided by the Nutrition Foundation, Inc.)

Serum gamma$_B$-globulin in normal adults and in rheumatoid arthritics before and during treatment with ACTH or cortisone. PHYLLIS W. FOSTER, WILLIAM Q. WOLFSON, MIOKO

EYA, WILLIAM D. ROBINSON, IVAN F. DUFF AND JOHN J. RICK (introduced by JOSEPH P. CHANDLER). *Rackham Arthritis Research Unit, Dept. of Internal Medicine, Univ. of Michigan Med. School, Ann Arbor.*

Serum gamma$_B$-globulin (*B*) denotes that serum sub-fraction precipitated by 13½% sodium sulfite, then estimated by an adaptation of previously reported micro-methods (details on request). Dauphinee and Campbell reported that considerable *B* was present in liver disease, but seldom in normals. In this study, 12 normal adults showed an average *B* equal to 3.5% (S.D. ±1.4%) of serum total protein. Prior to hormone treatment, 21 rheumatoids showed an average *B* equivalent to 8.3% (S.D. ±3.0%) of serum total protein. *B* concentrations show little correlation with Westergren sedimentation rate in untreated patients, but are definitely inversely correlated with true *A/G* ratios. In this series, an occasional rheumatoid showed either a normal Westergren sedimentation rate or a normal *B* concentration, but no patient had normal values for both indices. During ACTH or cortisone therapy, *B* concentrations, true *A/G* ratios, and Westergren sedimentation rates all change toward normal but to different extents and at different rates. The changes during hormone treatment and the comparative electrophoretic patterns of *B* and total gamma-globulin fractions will be illustrated.

Bacterial utilization of benzoyl amino acids and benzoylpeptides. SIDNEY W. FOX AND KENNETH F. ITSCHNER.* *Chemical Lab., Iowa State College, Ames.*

The abilities of *Lactobacillus arabinosus*, *Lactobacillus brevis*, and *Streptococcus faecalis R* to utilize benzoylamino acids in place of some of the corresponding essential amino acids have been studied. With *L. arabinosus*, the replacement was effective with benzoylleucine only. With *L. brevis*, only glycine was replaced, by hippuric acid. *S. faecalis R* could not use benzoylleucine, but it was able to employ benzoylmethionine instead of methionine for proliferation, although no other of the derivatives tested. *L. arabinosus* and *L. brevis* made slight use of dibenzoylcystine. In only one case was the benzoylamino acid inhibitory in full medium at the level tested; benzoyltryptophan inhibited the growth of *L. arabinosus* approximately 15%. When benzoylpeptides were tested for their ability to replace amino acids in the medium of *L. arabinosus*, the only ones which served this purpose contained terminal leucine residues. This limited utilizability contrasts to the generally wider nutritive value of unsubstituted peptides.

Determination of amino acid sequence of proteins. HEINZ FRAENKEL-CONRAT AND JANE FRAENKEL-CONRAT.* *Carlsberg Lab., Copenhagen, Denmark.*

Edman's ingenious method (P. EDMAN. *Acta Chem. Scand.* 4: 283, 1950) for the stepwise degradation of peptides has been adapted to use with proteins. The technique of addition of phenyl isothiocyanate to the amino groups remained unchanged. The thiohydantoin of the terminal amino acid, however, was split off in aqueous 0.6–1.2 N hydrochloric acid at 36°, in concentrated guanidine halide solution. After extraction with ether, it was determined spectrophotometrically near its absorption—maximum (267.5 mμ); the molar extinction coefficient of thiohydantoins is about 16,000. The nature of the terminal amino acid was established by paper chromatography after hydrolysis of the thiohydantoin with baryta according to Edman. The rest of the protein molecule was apparently not attacked under the above conditions, since it yielded a similar number of terminal amino acids, when again, and again, subjected to the same reactions. There appears to be no theoretical, and no major practical obstacle to the complete elucidation of the amino acid sequence of a peptide chain. With insulin, 3–3.5 end groups were found/12,000 MW-unit, with β-lactoglobulin about 2.5/mole. Recovery experiments with simple peptides indicate that corrections of the above values by 20% may have to be applied, which would bring them into accord with those of the literature. Qualitatively, the expected amino acids were found, but sometimes also alanine and glycine when these were not expected. In agreement with the DNP-method, low values (one end group/mole) were found for conalbumine and ovomucoid.

Spectrophotometric determination of cytochrome oxidase activity. WALTER J. FRAJOLA* AND CARL S. VESTLING. *Dept. of Chemistry, Univ. of Illinois, Urbana, and Dept. of Physiological Chemistry, Ohio State University, Columbus.*

Investigation of the rate of oxidation of reduced cytochrome *c* by brain and liver homogenates of rat or rabbit resulted in certain modifications of the spectrophotometric technique of Albaum, Tepperman, and Bodansky (*J. Biol. Chem.* 163: 641, 1946). Reduced cytochrome *c* solutions (Fe content about 0.30%) invariably exhibited appreciable auto-oxidation rates which were measured and corrected for in the procedure described. Studies of the reaction at various initial concentrations of reduced cytochrome *c* yielded data which are best explained by the combination first and zero order kinetic treatment recently reviewed by Neurath and Schwert (*Chem. Rev.* 46: 69, 1950). Heat-treated cytochrome *c* solutions showed greater reactivity than non-heated solutions. A comparison of the spec-

trophotometric procedure described and the manometric procedure indicated that the concentrations of cytochrome *c* considered to be sufficiently high to saturate the oxidase are much below saturation level. It is of interest that the behavior of an enzyme associated with particulate matter lends itself to the kinetic treatment described. The results are in accord with the view that there are limitations in the diffusion of reduced cytochrome *c* to and from the enzyme surface and in the orientation of the reduced substrate at active centers on the surface.

Quantitative measurement of blood (peroxidase) in stools. ALFRED H. FREE, MARION H. COOK* AND HELEN M. FREE.* *Miles-Ames Research Lab., Elkhart, Ind.*

Demonstration of occult blood in the stool is an important finding which may aid in the diagnosis of certain gastrointestinal disorders. Practically all tests for occult blood that are applied to stools make use of the fact that hemoglobin and its degradation products have a peroxidase activity. In order to arrive at a more satisfactory test for occult blood in the stool a quantitative method for measuring peroxidase activity of stools has been devised. This method involves dilution of the stool with water in a Waring Blendor. An aliquot of diluted stool is mixed with hydrogen peroxide and benzidine solution. This mixture after standing is diluted with acetic acid solution and the color measured in a spectrophotometer. Blood properly diluted and treated in the same way is used as a standard. Results are expressed as mg. hemoglobin/gm. of stool. A number of serial stool samples from normal subjects have been studied with this method. The effect of oral ingestion of various amounts of blood has been determined. After the oral ingestion of blood, the excess peroxidase appearing in the stool in the following 2 or 3 days represents one fourth–one half of the original activity of the blood. Correlation of the results of various qualitative occult blood tests with the quantitative results is being studied.

'Activation' of ascorbic acid oxidase by thyroxine and other amino acids. EARL FRIEDEN (introduced by KARL DITTMER). *Dept. of Chemistry, Florida State Univ., Tallahassee.*

The mechanism of the accelerating effect of thyroxine on the oxygen uptake of the ascorbic acid–ascorbic acid oxidase system first reported by Gemmill (*J. Biol. Chem.* 192: 749, 1951) has been investigated. The 'activation' of ascorbic acid oxidase appears to be a property of amino acids with thyroxine and structurally related amino acids effective at considerably lower concentrations than the simpler amino acids. DL-thyroxine, 3×10^{-5} M, effected seven- to tenfold elevations of the rate of ascorbic acid oxidation in the presence of ascorbic acid oxidase, while approximately 10^{-2} M phenylalanine, glycine, and other amino acids were required for similar effects. Thyroxine reverses and prevents the inactivation of ascorbic acid oxidase caused by dilution, oxygenation, or heat. No effect of thyroxine is observed if a protective protein, such as gelatin (POWERS, LEWIS AND DAWSON. *J. Gen. Physiol.* 27: 167, 1944) is added to the enzyme prior to inactivation. Using a standard dilution procedure, it was possible to relate the oxygen consumption of the system to the thyroxine concentration. The structural specificity of thyroxine in this reaction has been studied using the compounds of Frieden and Winzler (*J. Biol. Chem.* 176: 155, 1948; 179: 423, 1949). 0-Benzyl-3,5-diiodo-DL-tyrosine, a thyroxine antagonist, was almost as effective as thyroxine, but the corresponding N-acetyl compound was inactive at 10^{-3} M. The thyroxine-like compound, 3,5-diiodo-4-(3′,5′-diiodo-4′-hydroxyphenoxy)-benzoic acid, was inactive at 10^{-3} M. The data suggest that a free amino group is required for the effect on ascorbic acid oxidase activity.

Hormonal effects on the incorporation of labeled amino acids in tissues of the reproductive tract. EDWARD H. FRIEDEN, A. C. STEELE* AND M. A. TELFER.* *Biological Labs., Harvard Univ., Cambridge, Mass.*

The effects of steroid hormones and relaxin upon the incorporation of labeled amino acids into proteins of reproductive tract tissues of guinea pigs and rats have been studied *in vitro* and *in vivo*. Symphyseal slices from guinea pigs treated with estrogen for 4–7 weeks incorporate glycine-1-C^{14} *in vitro* 5–10 times as rapidly as does guinea pig liver. Upon fractionation of the connective tissue proteins, appreciable activity is found in the collagen component. One week after withdrawal of estrogen, the observed incorporation rate has decreased to 25% of its initial value. Symphyses from untreated castrate animals show only slight activity. Four days after the injection of a single dose of glycine, the specific activities of proteins of the uterus and symphysis approximate those of serum and visceral organs in estrogen treated guinea pigs. In castrate animals, or in those in which treatment with estrogen has been discontinued, activities of both uterine and symphyseal proteins are reduced. Relaxation has no apparent effect on the activity of symphyseal protein. The uterus of the castrate rat incorporates glycine approximately 5 times as rapidly as rat liver *in vitro*. Following a single injection of 5γ estradiol, the rate increases, reaching a maximum in 48 hours. In contrast, the uptake of DL-

alanine-1-C^{14} is reduced by estrogen treatment. The incorporation of both glycine and alanine in uteri of rats treated with progesterone is somewhat greater than in castrate controls. (Supported by a grant from the U. S. Public Health Service to F. L. Hisaw).

Enzymatic synthesis of several uncommon ribosides and desoxyribosides. MORRIS FRIEDKIN. *Dept. of Pharmacology, Washington Univ., St. Louis, Mo.*

The enzymatic release of inorganic phosphate according to the Kalckar reaction, ribose-1-phosphate + base ⇄ ribose-1-base + inorganic phosphate (*J. Biol. Chem.* 167: 477, 1947), can be used as an indication of nucleoside synthesis. When incubated with purified preparations of purine or pyrimidine nucleoside phosphorylase from horse liver and ribose-1-phosphate or desoxyribose-1-phosphate, the following bases gave the Kalckar reaction: 8-azaguanine, 2-thiouracil, 5-aminouracil, and other substituted uracils (5-iodo-, 5-bromo-, 5 chloro-, 5-nitro-, 2-thio-5-iodo-, and 2-thio-5-bromo-). Evidence for the enzymatic formation of the ribosidic analogue of thymidine was similarly obtained. Paper chromatography was used to substantiate the phosphate data. The ribo- and desoxyribo-nucleosides of 8-azaguanine, synthesized by the Kalckar reaction, were isolated in pure crystalline form. The microbiological activities of these nucleosides are now being investigated. Among the compounds tested which gave a negative Kalckar reaction were: 5-methylcytosine, orotic acid, ureidosuccinic acid, dihydrothymine, barbituric acid, arginine, histidine, histamine, citrulline and ornithine. (Supported by a research grant from the NIH, Public Health Service.)

Purine and pyrimidine metabolism of normal and phage-infected *Escherichia coli* B. SAMUEL FRIEDMAN AND JOSEPH S. GOTS (introduced by OTTO ROSENTHAL). *Dept. of Microbiology, Med. School, Univ. of Pennsylvania, Philadelphia.*

Previous findings from this laboratory have shown that resting cell suspensions of *E. coli* B exhibit deaminase activity for adenine, adenosine, adenosine-3-phosphate, isoguanine, 2,6-diaminopurine, cytosine, cytidine, and cytidylic acid; nucleosidase activity for inosine, guanosine, uridine and thymidine; and nucleotidase activity for inosinic, uridylic and guanylic acids. Infection of such cells with T4r+ bacteriophage did not obliterate these enzymatic activities but showed significant differences. Under growth conditions in salt-glucose media leading to active synthesis of desoxypentose nucleic acid, the behavior of these two systems with adenine, or thymine and their derivatives is different. With adenine or adenosine as substrates, disappearance occurs to a greater extent in infected cells with more hypoxanthine accumulating. The normal system reutilizes this reaction product. A reproducible initial lag in deamination of adenine can be demonstrated in the infected system. Neither adenosine-3-phosphate nor desoxyadenylic acid are deaminated. Similarly, infected systems utilize thymine, thymidine and thymidylic acid to a greater extent than the normal. With the normal system thymidine is split to the base, the desoxypentose rapidly being utilized and the base disappearing slowly. Both the split of thymidine to the base and the disappearance of desoxypentose lags appreciably in infected systems.

Adaptation of *Achromobacter fischeri* to fructose utilization. STANLEY FRIEDMAN (introduced by W. D. MCELROY). *Dept. of Biology, Johns Hopkins Univ., Baltimore, Md.*

A marine microorganism, *Achromobacter fischeri*, exhibits good growth on a completely synthetic medium containing an inorganic nitrogen source and 0.5% glycerol or glucose as a carbon source. If 0.5% fructose is used as a carbon source, in place of the above, the bacterium will not grow unless any one of a large number of amino acids is added to the medium. These amino acids, the most efficient of which are glutamic acid, histidine, and arginine, are effective in initiating growth in concentrations as low as 0.02 mg/20 ml. of medium, and once growth is started, it continues to a maximum, regardless of the amount of amino acid added. The organism cannot use these amino acids as the sole source of carbon in the medium. In an effort to determine whether or not the enzymes concerned with the primary stages of fructose metabolism were present in the glycerol-grown cells, cell-free extracts were made, and the enzymes thus isolated were tested. It was found that a very active zwischenferment was present, so using the reduction of TPN as a criterion of enzymatic activity, fructose-6-phosphate, and glucose or fructose + ATP were tested. All of these substrates, when added to the extract, caused a marked reduction of TPN. Comparisons made between adapted and unadapted cells showed very little, if any, difference in the relative activities of the hexokinases present. It can, therefore, be concluded that the metabolic block to fructose utilization is not concerned with primary phosphorylative reactions. Some possible mechanisms of the origin of the block will be discussed.

Formate production in the intact rat. BERNICE FRIEDMANN* AND SIDNEY WEINHOUSE. *Lankenau Hosp. Research Inst. and Inst. for Cancer Research, Philadelphia, Pa.*

A method of estimating formate production in the intact rat has been developed, which consists

in administering a labeled compound by intraperitoneal injection together with a dose of 2 mM of unlabeled sodium formate. During the ensuing 3-6 hours approximately half of the administered formate is excreted in the urine. Any metabolic formate is 'trapped' and excreted with the administered acid, and the amount arising from the labeled carbon can be estimated by assaying the excreted formate for its radioactivity. The reliability of the method is attested by the fact that carbon atoms which from earlier studies are known or can be inferred to yield formate, viz., the α carbons of glycine, glycolate and glyoxylate, the β carbon of serine and the methyl groups of acetone, sarcosine and methionine, all yield formate. Glycine and glycolate carboxyls, and acetate methyl did not yield formate. Formate production was particularly high from the methyl groups of acetone, sarcosine and methionine, the conversion being about 10 times that from the glycine α carbon at similar injection levels. In a variation of the above procedure, C^{14}-labeled formate was administered and approximate estimations of the minimal over-all rate of formate production calculated from the extent of isotopic dilution of the excreted formate. Both fed and fasted normal rats produced formate at a fate estimated at about 30–60 μM/hr/100 gm. of body weight. Formate oxidation was very rapid, approximately 80–90 % of the activity not excreted as formate having been excreted as CO_2 in less than 5 hours.

Testosterone production in a normal man. David K. Fukushima,* Konrad Dobriner and T. F. Gallagher. *Sloan-Kettering Inst. for Cancer Research, New York City.*

An estimate of the maximum daily testosterone production of a normal adult man was made from the following experiment. The subject received 11 mg. of testosterone, stably labelled with deuterium, by slow intravenous infusion for 8 hours. Urine collections were made during this time and for 1 hour after cessation of the infusion. The total amount of testosterone metabolized was determined from the isotopic dilution after addition of carrier followed by the isolation of androsterone and etiocholanolone in pure form. It was found that 37% of the administered testosterone was metabolized and excreted during these 9 hours. During several control studies this subject excreted androsterone and etiocholanolone in the total amount of approximately 12 mg/24 hours. If one assumes that testosterone is continuously produced at a steady rate, the percentage recovered during the infusion experiment is a measure of daily production. Therefore, this subject's testosterone secretion was approximately 30 mg/24 hours. Since hormones other than testosterone are metabolized to androsterone and etiocholanolone, this estimate is a maximum figure. The fate of the labelled hormone was followed for 48 hours. During the first day 48% was excreted and in the following 24 hours 2% was excreted as androsterone and etiocholanolone.

Effect of Dibenamine on response of arterial smooth muscle to epinephrine, nor-epinephrine, electrical stimulation and anoxia. Robert F. Furchgott. *Pharmacology Dept., Washington Univ. School of Medicine, St. Louis, Mo.*

Spirally cut strips of rabbit aorta suspended in oxygenated Krebs solution at 37.5° give a minimal detectable contraction with concentrations of epinephrine of the order of 10^{-9}. Sensitivity to nor-epinephrine is approximately the same. Dibenamine (10^{-6}) can completely block the contractile response to even high concentrations of epinephrine and nor-epinephrine. After Dibenamine, *relaxation* with epinephrine can frequently be demonstrated if a state of tonic contraction is first produced by histamine or acetylcholine. No relaxation has been obtained with nor-epinephrine under similar conditions. Dibenamine also partially inhibits the contractile response to hitamine. Application of an alternating current for about 10 seconds causes a relatively fast contraction during current flow, followed after cessation of current by a further slower contraction and prolonged slow relaxation. The contraction after current flow is probably due to the liberation of an epinephrine-like substance in the artery during electrical stimulation, since an alternating current after Dibenamine produces only the relatively fast contraction, followed immediately by a faster relaxation than occurs without Dibenamine. Contractile response to epinephrine is about 50% lower anaerobically than aerobically with glucose (0.01M) present. Without glucose it is over 90% lower, probably because of deficient glycolytic energy. Following a period of anoxia without glucose, a temporary contraction occurs on reintroduction of oxygen. This contraction has been attributed to an epinephrine-like substance liberated during anoxia, since it does not occur with Dibenamine-treated arteries. (A grant from the Life Insurance Medical Research Fund supported this study.)

Catabolism of malononitriles in normal and tumor-bearing animals. Emery M. Gal,* Fong-Han Fung* and David M. Greenberg. *Div. of Biochemistry, Univ. of California School of Medicine, Berkeley.*

The observation that certain substituted malononitriles produce retardation of the growth of transplant tumors in mice (*J. Am. Chem. Soc.* 73: 502, 1951) led us to investigate the catabolism of the nitriles in normal and tumor-bearing ani-

mals. The injection of nitriles is followed by an increased urinary excretion of thiocyanate, which is not quantitative. Variations in the amount of thiocyanate excreted are due to differences in catabolic pathways rather than to the rate of conversion into cyanide. Formation of thiocyanate from cyanide is through the enzyme, trans-sulfurase. We have determined the trans-sulfurase content of the tissues of various normal and tumor-bearing animals in relation to the nitriles. The trans-sulfurase activity of tumors were found to be much less than that of liver and kidney, but by no means insignificant. The activity of the normal liver tissue is unaffected, whereas the activity of the hepatoma decreases upon continued azo dye administration. Tissues of Walker carcinoma-bearing rats showed decrease of trans-sulfurase activity. Daily injections with malononitriles for 3 weeks did not affect the trans-sulfurase activity of the tissues of C3HS-bearing mice. Study of kinetics of the trans-sulfurase reaction in tissue homogenates revealed that the malononitriles do not serve as substrates, nor do they inhibit the activity of this enzyme or competitively displace cyanide as the substrate. The process of detoxication of nitriles seems to be mediated by two enzyme systems, one responsible for the carbon-carbon bond breakage, releasing cyanide, the other, trans-sulfurase, converts cyanide into thiocyanate.

Seasonal variation in toxicity of Demarol in white mice. ROBERT E. GALLOWAY* AND EMMETT B. CARMICHAEL. *Biochemistry Dept., Med. College and School of Dentistry, Univ. of Alabama, Birmingham.*

Young adult white mice, weighing 25 gm. or more, were used in toxicity studies with Demerol hydrochloride (Isonipecaine). A fresh aqueous solution was injected intraperitoneally. The mice received only a single dose each. The doses varied by 5 mg. increments from 75–100 mg/kg. for 177 mice used during the summer months and from 115–145 mg/kg. for 106 mice during the winter months. The smallest dose to cause death was 75 mg/kg. during the summer months and 115 mg/kg. during the winter months. The LD_{50} of Demarol hydrochloride during the summer months seems to be in the range of 80–95 mg/kg. while the LD_{50} for mice during the winter months seems to be about 130–135 mg/kg.

Absence of correlation between vitamin A alcohol in liver and plasma in rats. J. GANGULY,* N. I. KRINSKY,* AND HARRY J. DEUEL, JR. *Dept. of Biochemistry, Univ. of Southern California, Los Angeles.*

A recent report by Ganguly *et al.* (*Arch. Biochem. Biophys.* In press, Jan. 1952) indicates that different specific proteins are involved in the transport of vitamin A ester and vitamin A alcohol in the rat and chicken. Glover *et al.* (*Biochem. J.* 41: 97, 1947) reported that a direct relationship obtains between vitamin A alcohol in the liver and blood of rats. Experiments were carried out on 114 male and 14 female rats which were receiving Purina diet or a vitamin A-deficient diet. The latter group was supplemented with vitamin A ester in single doses of 10,000, 25,000 and 50,000 or 25,000 IU/day for 13 days. The rats were sacrificed at least 5 days after the last dose of vitamin A supplement and after a 12-hour fast. In all samples, vitamin A alcohol and ester were separated chromatographically for analysis. The alcohol alone was determined in blood and both ester and alcohol in the liver. No correlation could be noted between plasma and liver vitamin A alcohol. We believe that the level of plasma vitamin A alcohol is maintained by a specific protein carried. (Supported by grants from The Nutrition Foundation and Procter and Gamble Co.).

Collagen synthesis in tissue cultures. HORACE W. GERARDE AND MARION JONES (introduced by H. A. MATTILL). *Depts. of Biochemistry and Bacteriology, College of Medicine, State Univ. of Iowa, Iowa City.*

A quantitative roller-tube method was used to measure the rates of collagen synthesis in cultures of embryonic chick heart, lung and intestine. Each culture tube contained an accurately weighed quantity of tissue fragments (20–40 mg. wet weight) explanted on the inner surface of the tube in a thin film of chicken plasma. Collagen was determined by colorimetric estimation of hydroxyproline in acid hydrolysates of protein extracted from the tissue by autoclaving. The initial quantity of collagen in the culture tubes was calculated from the known wet weight of tissue and previously established values for μg. of collagen/mg. tissue. Following cultivation at 37° the contents of 30–40 tubes were pooled, dehydrated with acetone, dried to constant weight at 108° and analyzed for collagen. Many different culture media were tested (proteoses, peptones, acid and enzymatic digests of fibrin, amino acid solutions, extracts of adult tissues) but a net synthesis of collagen was obtained only with an extract of embryonic tissue. Collagen production was most rapid in lung cultures, which yielded an average of 384 μg. from an average initial 58 μg. of collagen/culture tube after a 10-day cultivation period. A threefold increase in collagen was obtained with heart, and a doubling with intestine in 10 days.

Acyl-coenzyme A deacylase. J. GERGELY, M. P. HELE AND C. V. RAMAKRISHNAN (introduced by F. M. STRONG). *Inst. for Enzyme Research, Univ. of Wisconsin, Madison.*

Green and Beinert (*Phosphorus Metabolism,*

Vol. I, Johns Hopkins Press, 1951) have shown that the interaction of the pyruvic and α-ketoglutaric oxidases with DPN requires the presence of both coenzyme A and an enzymic factor (F). F has now been purified from extracts of pig heart some 60 times by salt fractionation and adsorption on tricalcium phosphate gel. Reduction of DPN can be followed spectrophotometrically when the soluble α-ketoglutaric oxidase of Sanadi and Littlefield is supplemented with CoA (catalytic amounts) and F. This reaction has been used as the basis of a rapid assay system for CoA (R. Von Korff). The evidence that F functions as a deacylase is threefold. *1)* It catalyzes the breakdown of succinyl-coenzyme A, as shown by the disappearance of the hydroxamic acid reaction and the liberation of free coenzyme A. *2)* It will replace arsenate in the breakdown of acetyl phosphate catalyzed by transacetylase and CoA. *3)* Transacetylase, arsenate, and coenzyme A will bring about the spectrophotometric reduction of DPN by the soluble pyruvic oxidase of Jagannathan and Schweet in the absence of coenzyme A deacylase. Whether the same enzyme is involved in the deacylation of both acetyl and succinyl CoA is still an open question.

Potassium and effect of pyruvate on fatty acid oxidation in vitro. ROBERT P. GEYER, ETHEL C. BOWIE AND JACQUELINE C. BATES (introduced by F. J. STARE). *Dept. of Nutrition, Harvard School of Public Health and Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.*

Slices of rat liver were incubated with carboxyl-labeled octanoic or palmitic acids in media of varying potassium concentration. High potassium concentrations caused a greatly increased labeled acetoacetate production from either substrate without increasing $C^{14}O_2$ formation. Lithium behaved similarly to K in this respect. The addition of pyruvate in the case of low-K media caused a marked reduction in the oxidation of palmitic acid, but not of octanoic acid. In the case of high-K media, however, pyruvate caused a decrease in the oxidation of both fatty acids. Studies have also been done with rat kidney slices and the results are similar to those obtained with liver, except that the $C^{14}O_2$ formation as well as acetoacetate production was affected by the K concentration of the medium. These results with pyruvate show that fatty acid metabolism is influenced by carbohydrate and support earlier findings obtained *in vivo* in which glucose was found to decrease palmitate oxidation by the rat.

Crystalline rat liver lactic dehydrogenase. DAVID M. GIBSON, EDWIN O. DAVISSON AND BIMAL K. BACHHAWAT (introduced by CARL S. VESTLING). *Dept. of Chemistry, Univ. of Illinois, Urbana.*

Isolation and crystallization of rat liver lactic dehydrogenase has been achieved through a series of low temperature ethanol and ammonium sulfate precipitations. Liver was homogenized in 0.5 M NaCl and then brought to 16% ethanol concentration by weight at −5° (pH 6.5). After centrifugation the clear supernatant solution was dialyzed against 0.5 M NaCl at 0°. The enzyme was precipitated by adding solid ammonium sulfate to a concentration of 0.3 gm/ml. The precipitate was suspended in water, dialyzed against 0.03 M NaCl and then brought at −3° to 5% ethanol, ionic strength 0.03, with phosphate buffer (pH 5.8). After centrifugation the enzyme was recovered from the supernatant liquid by bringing the ethanol concentration to 30%. The precipitate was redissolved in phosphate buffer (ionic strength 0.05, pH 7.7) and then reprecipitated at −8° with 30% ethanol. This step was repeated with a final ethanol concentration of 20% at a higher enzyme concentration. The final enzyme precipitate was extracted several times with phosphate buffer to remove certain impurities. The residue was dissolved in 1.0 M ammonium sulfate at pH 7. Crystallization resulted in a very concentrated enzyme solution upon introducing ammonium sulfate by dialysis. The final crystalline preparation was essentially homogeneous in the ultracentrifuge.

Further studies in fat and carbohydrate metabolism using C^{14}-labeled glycerol. L. I. GIDEZ* AND M. L. KARNOVSKY. *Dept. of Biological Chemistry and Biophysical Lab., Harvard Med. School, Boston, Mass.*

An attempt to measure the degree of lipolysis that occurs during the absorption of triglycerides has been made. Triglycerides labeled in the glycerol moiety were administered orally to rats, and the expired CO_2 counted. By relating the total number of counts expired at any given time to a reference curve established for free radio-glycerol, a measure of lipolysis was provided. For tributyrin, it was estimated that from 55–90% was split in 2 hours and 100% in 4 hours; for triolein, from 35–55% was split in 2 hours, and from 40–100% in 4 hours. The results are based on the assumption that free glycerol only is oxidized, and the possibility of exchange reactions between glycerides and glycerol is not excluded. In another series of experiments, animals were despatched at different times after the administration of radio-glycerol. Blood sugar and liver glycogen reached peak activity after 1 hour; at this time the amount of liver glycogen derived from glycerol was 15–20%, and the fraction of administered glycerol converted to glycogen about 5%. The liver neutral fat reached peak activity in 30 min-

utes and the phosphatides at about 2 hours. The activity curves of these components crossed at about 90 minutes. The glycerol, fatty acid and steroid components of neutral fat, and the glycerophosphoric acid and fatty acid moieties of phosphatide have been individually counted.

Inhibition of esterase by N-substituted amides. ANDREW J. GLAID* AND G. W. SCHWERT. *Dept. of Biochemistry, Duke Univ. School of Medicine, Durham, N. C.*

In previous investigations in this laboratory, the specific esterase activity of the pancreatic proteases has been investigated in detail. The reciprocal problem, i.e. whether a typical esterase can also hydrolyze substrate analogs containing a substituted amide group in place of the ester group has been investigated with horse liver esterase (prepared by the method of CONNORS, PIHL, DOUNCE, AND STOTZ. *J. Biol. Chem.* 184: 29, 1950) and N-methyl and N-ethyl butyramide. Neither of these amides is hydrolyzed by liver esterase. This result is consistent with the well-established observation that specific esters are hydrolyzed by the pancreatic proteases at much higher rates than are the corresponding amides and peptides. Both amides are, however, competitive inhibitors of liver esterase, the values of K_I being about 15 times as large as the values of K_m for the corresponding esters. The inhibition constants have been determined at 3 temperatures between 10° and 35° and the thermodynamic constants for the inhibition process have been estimated.

Human serum lipoprotein functions in starvation lipemia. WILLIAM H. GOLDWATER, J. R. SNAVELY, MALCOLM L. RANDOLPH AND ROY H. TURNER (introduced by WILLIAM B. WENDEL). *Depts. of Medicine and Biophysics, Tulane Univ. School of Medicine, New Orleans, La.*

Starvation lipemia has been studied in normal male subjects, in an attempt to describe serum lipoprotein mechanisms involved. Increases of all whole serum lipids occurred, neutral fat predominating on a concentration basis, with considerable percentage rises also in free cholesterol. Quantity ultracentrifugation and subsequent chemical analysis of successive levels of the centrifugates (*J. Clin. Investigation* 30: 1071, 1951) were used to study lipoprotein responses involved. Lipid changes in the top, or cream, layer were generally upward after starvation. Predominant were the increases after starvation of all lipids at 1.010 specific gravity, in the third level. Cholesterol ester and phospholipid decreases in the lower half of the centrifugate suggest participation of denser lipoproteins in the constitution of lighter complexes. Neutral fat increments in lower levels indicate a response to increased fat carriage by denser lipotroteins. The hypothesis is proposed that some of the low-density lipid complexes function in lipid transport from depots to the liver, while those in the denser centrifugate levels represent lipids moving from the liver to tissues or depots.

Adaptive enzyme formation in the chick embryo. MALCOLM W. GORDON (introduced by PHILIP P. COHEN). *Dept. of Physiological Chemistry, Univ. of Wisconsin, Madison.*

The yolk sacs of 5- to 10-day chick embryos were injected with 25–75 mg. adenosine. After varying periods of incubation, subsequent to this treatment, the eggs were opened and the adenosine deaminase activity of the whole embryo or the embryonic liver was determined by the spectrophotometric method of Kalckar. In untreated eggs, adenosine deaminase and xanthine oxidase are not demonstrable in the liver until some 24–48 hours after hatching, even though the former enzyme can be shown in whole embryo homogenates as early as the 7th day after laying. Following the above treatment, however, considerable adenosine deaminase activity is present in the liver 2–5 days prior to hatching, with the increase in adenosine deaminase titer in whole homogenates being accounted for by the increase in the liver enzyme. Data will be presented demonstrating some threefold increased activity in the liver following repeated small injections of adenosine compared to one large injection. The parallelism between this phenomenon and antibody formation as well as the possible significance of adaptive enzyme formation in morphogenesis will be discussed.

Isolation of a crystalline protein from milk. WILLIAM G. GORDON AND WILLIAM F. SEMMETT.* *Eastern Regional Research Lab., Philadelphia, Pa.*

In 1939 Sørensen and Sørensen (*Compt. rend. trav. du lab. Carlsberg, Sér. chim.* 23, No. 7) isolated from milk whey a crystalline protein which they designated 'insoluble substance.' The protein was obtained from the crude β-lactoglobulin fraction. In crystalline form the 'insoluble substance' appeared identical with β-lactoglobulin but it differed from the latter in its insolubility in dilute salt solution (0.1 M NaCl) and in its higher tryptophan content. The new protein was not characterized further and its presence in whey has not been generally recognized. We have succeeded in isolating crystalline 'insoluble substance' along the lines indicated by the Sørensens and have confirmed many of their observations. We have found the protein to be electrophoretically homogeneous at pH 8.4 (mobility = -4.2×10^{-5} cm²./volt/sec., veronal buffer, ionic strength = 0.1). At pH 3.3, however, two components are

present (mobilities = +5.7 and +7.3, lactate buffer, ionic strength = 0.1).

Isolation and identification of fatty acids in milligram quantities as hydroxamic derivatives. ALEXANDER GOUREVITCH AND JOSEPH LEIN (introduced by W. W. WESTERFELD). *Dept. of Zoology, Syracuse Univ., Syracuse, N. Y.*

Hydroxamic acid derivatives of fatty acids are prepared by reacting neutral fats or fatty acid esters with an alcoholic solution of hydroxylamine hydrochloride and methoxide (LEWIS. *Bioch. J.* 20: 1356, 1926). The hydroxamic acids of greater degree of unsaturation are more soluble in aqueous methanol than those of lesser degree of unsaturation. By increasing the water content of aqueous methanol, fractions can be precipitated showing a progressive increase in unsaturation. Preparation and fractionation of these derivatives are carried out at room or lower temperatures, thus reducing the possibility of autoxidation or isomerization of fatty acids. The fractions can be estimated quantitatively by measuring light absorption of their ferric complexes at 530 mμ. The different hydroxamic acids have the same molar absorption (HILL. *Indust. & Eng. Chem. Anal. Ed.* 18: 317, 1946). The qualitative composition of the fractions is determined by reverse phase paper partition chromatography. Paper with affinity for nonpolar solvents is prepared by treating filter paper with silicone resin (KRITCHEVSKY AND TISELIUS. *Science* 144: 299, 1951). Benzene is used as the stationary phase, and aqueous alcohols, saturated with benzene, as the mobile phase. After development, paper is spotted with ferric chloride solution. The more unsaturated derivatives give higher R_F values. The spectrophotometric determination and the chromatographic analysis can be carried out on less than 100μg. of fatty acid derivative.

Heme components of cytochromes of horse heart. S. GRANICK. *Rockefeller Inst. for Med. Research, New York City.*

The heme components of the cytochromes have been isolated from acetone-HCl extracts of 200 lbs. horse heart. (Cytochrome *c* is presumably not extracted.) The Fe of the hemes was removed to convert them to their respective porphyrins for more ready separation. Porphyrin *a*, derived from cytochrome *a*, has an HCl no. ~15.5 and is extractable from ether with M/15 PO_4 buffer pH 7.2–7.5. The rhodo type spectrum of this porphyrin is due to the presence of a formyl group side chain. The formyl group was demonstrated by conversion of the porphyrin to the oxime, nitrile, thioacetal and dimethyl acetal derivatives which have characteristic absorption spectra. A monovinyl side chain is also present, as revealed by the absorption spectrum of porphyrin *a* after its formyl group was reduced by Al isopropoxide, or after the formyl group was converted to the dimethyl acetal. Reduction with Pd + H_2 of the vinyl group resulted in the expected 4 mμ shift to the blue of the absorption bands of the porphyrin thus supporting the presence of one vinyl side chain. Titration of porphyrin *a* revealed 3 weakly acidic groups, presumably all carboxyl. Insertion of iron into porphyrin *a* forms a reconstructed heme *a* from whose iron content a molecular weight of ~940 is estimated. Analyses indicate porphyrin *a* to possess some 19 C atoms more than protoheme; these C atoms are not present in a group in ester linkage. In addition to porphyrin *a* and protoporphyrin several other porphyrins have been found. From the mixture of porphyrins having an HCl no. below 2, five porphyrins have been isolated by counter current distribution and crystallized. One has been identified as hematoporphyrin, another possesses one hydroxyethyl side chain, and another contains a single vinyl side chain.

Attempts to prevent or reduce radiation injury by pharmacological means. JOHN L. GRAY,* ELIZABETH J. MOULDEN,* JOHN T. TEW* AND H. JENSEN. *Army Med. Research Lab., Fort Knox, Ky.*

A protective effect of Pitressin and epinephrine against local x-irradiation injury of the leg of the rat has been demonstrated by Pendergrass and associates (personal communication). Extending these observations a study was made of the possible protective effect of these agents against total body x-irradiation. Male, Sprague-Dawley rats (220–290 gm.) were irradiated in pairs, one untreated animal serving as control and the other receiving either 5 units of Pitressin or 0.2 mg. of epinephrine intraperitoneally at varying intervals before or after x-irradiation (880r). Survival rates apparently were increased significantly by pre-irradiation injection of pitressin at 5, 20 or 40 minutes prior to exposure. Pitressin given postirradiation produced no significant protection. Epinephrine injection at 5 or 20 minutes before irradiation appeared to afford definite protection. The protective effect of these principles may be due to their property of producing a temporary tissue anoxia. The possible significance of the amount of oxygen in the tissue to radiation effects was further investigated by studying the possible protective effect of other vasoconstrictive agents and of various methemoglobin producing substances. The possible mechanisms of the ameliorating influence of these compounds and the correlation between degree of methemoglobin formation and protection against radiation injury will be discussed.

Activation of acetate, acetoacetate and fatty acids in soluble extracts of pig heart. D. E. GREEN, H. BEINERT,* D. GOLDMAN,* R. VON KORFF* AND S. MII.* *Inst. for Enzyme Research, Univ. of Wisconsin, Madison.*

The fractionation, purification and assay of the enzymes involved in the activation of acetate, acetoacetate (β-ketoacids) and fatty acids will be described. ATP (ADP), CoA and magnesium ions are essential components of these systems. Evidence will be presented bearing on the following reactions currently postulated: *1*) phosphorylation of CoA by ATP (Lynen, Green); *2*) interaction of phosphoryl CoA with acetate, acetoacetate and butyrate; *3*) conversion of acetoacetyl CoA to 2 molecules of acetyl CoA (Lynen, Lipmann); *4*) conversion of butyroacetyl CoA to 1 molecule of acetyl CoA and 1 molecule of butyryl CoA; and finally *5*) oxidation of butyryl CoA to acetoacetyl CoA.

An electrotitrimetric method for measuring the enzymatic hydrolysis of ATP. IRVING GREEN AND ROBERT G. PARRISH (introduced by W.F., H. M. MOMMAERTS). *Dept. of Biochemistry Duke Univ. School of Medicine, Durham, N. C.*

In the hydrolysis of ATP to ADP by myosin-ATPase, phosphoric acid is produced. By the stepwise addition of standardized base, it is possible to maintain the initially adjusted pH constant throughout the reaction, thereby determining the amount of base used as a function of time. In order to express this in terms of moles of phosphate produced, it is necessary to know the relationship between the latter and the number of equivalents of base utilized. Since this relationship is a complex function of pH and ionic composition of the reaction medium, a comprehensive study has been undertaken to determine its dependency upon the concentration of various ions throughout the pH range 6–10. Alberty *et al.* (*J. Biol. Chem.* 193: 425, 1951) have developed an equation which gives the valence of the reaction ATP to ADP plus P as a function of the pH and of the pK's of ATP, ADP and phosphoric acid, determined by them in 0.15 M NaCl. This valence plotted against pH gives a typical sigmoid titration curve. In the neighborhood of pH 9, one equivalent of acid appears/mole of phosphate produced, whereas below pH 6 no acidification occurs. In the present work, the acid produced and the phosphate liberated were determined analytically in the same samples. The ratios thus obtained, when plotted against pH, also yielded sigmoid curves between the same limits (0–1 equivalent of acid) from pH 6.5–9.0, but these curves are considerably displaced toward the alkaline side, with respect to Alberty's curve. This indicates that various interactions among ATP, ADP, phosphoric acid and other ions can significantly alter the effective pK's of the above components of the reaction. The data so obtained permit the determination of the enzymic hydrolysis of ATP by titration with standard base to keep the pH constant. The method has been developed in order to reinvestigate the actomyosin-ATPase system, and a number of results will be presented.

Further purification and chemistry of coenzyme A. J. D. GREGORY (introduced by G. D. NOVELLI). *Biochemical Research Lab., Massachusetts General Hosp., Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.*

During the isolation of coenzyme A (CoA), a puzzling problem was the high sulfur content of some 60–80% pure preparations, amounting to nearly 2 sulfurs/pantothenic acid. On paper chromatography of acid hydrolysates, 2 sulfur compounds appeared, one identified as 2-mercaptoethylamine disulfide and the other as a mixed disulfide of cysteine and 2-mercaptoethylamine. In view of the identification by Snell and his group of N-pantothenyl 2-mercaptoethylamine (*Lactobacillus bulgaricus* factor, pantetheine) as a component of CoA, the large proportion of cysteine found in some preparations was now concluded to be a contaminant which had been linked to reduced CoA through formation of a disulfide bridge by simultaneous oxidation during isolation. Separation of the cross-linked cysteine from CoA was obtained by including a reduction step in the prufication procedure. Starting with CoA of 20% purity or better, reduction with zinc and hydrochloric acid was followed by precipitation of the mercury salt. This was decomposed with hydrogen sulfide and the CoA adsorbed on Duolite CS-100 resin from .2N hydrochloric acid solution. Elution with water and freeze-drying gave a nearly pure product in 15% yield. This assayed 360–400 U/mg. (estimated for pure CoA, 413 U/mg.), with the following analytical data: pantothenic acid 26.8% and ratios to this of adenine 1.05, total phosphorus 2.83, monoesterase-labile phosphorus 0.96, and sulfur 1.07. Theory for molecular weight of 767 (pantothenic acid, 2-mercaptoethylamine, adenosine, 3 phosphorus, minus 5 waters) is pantothenic acid 28.6%.

Further studies in citrulline biosynthesis. SANTIAGO GRISOLIA* AND PHILIP P. COHEN. *Dept. of Physiological Chemistry, Univ. of Wisconsin, Madison.*

We have shown previously 2 main steps in the enzymatic synthesis of citrulline from ornithine in the presence of carbamylglutamate. The enzymatically formed carbamylglutamate derivative (compound X) decomposes rapidly ($t_{\frac{1}{2}}$ at

38°, pH 7.3, 46.2 minutes). Earlier attempts to separate compound X from the components of incubation mixtures using several methods met with relatively little success. Best results were obtained with paper chromatography at 2° using 60% isopropyl alcohol. Efforts recently have been directed toward finding optimum conditions both for maximum accumulation and isolation of compound X. Using these conditions compound X separates in good yields by fractionation with barium and alcohol. Total purification or structural identification has not been achieved as yet. However preparations practically free of nucleotides, ammonia and inorganic phosphate have been obtained which upon acid hydrolysis liberated a mole of inorganic phosphate/mole of compound X. Enzyme complexes I and II have been only partially purified; however preparations of enzyme II free of enzyme I can condense compound X and ornithine to form citrulline at the rate of 2000 μM/mg. nitrogen/hour at 38°. There appears to be at least 50 times more activity for enzyme II than for enzyme I in liver. As a consequence there is no accumulation of compound X in crude enzymatic mixtures catalyzing the overall reaction ornithine → citrulline. Under suitable conditions 50 μM of citrulline can be synthesized/μM of carbamylglutamate.

Interaction of salts with protein crystals. M. L. Groves,* N. J. Hipp* and T. L. McMeekin. *Eastern Regional Research Lab., Philadelphia, Pa.*

Protein crystals are permeable to small molecules such as salts and organic substances. It has been assumed that the liquid of crystallization is composed of 2 kinds of water; thus, water which is bound to the protein and is not available as a solvent ('bound water'), and water through which ions diffuse freely and which has the same composition as the suspending liquid. (Perutz, M. F. *Tr. Faraday Soc.* XLII B: 187. 1946). Composition studies on large single β-lactoglobulin crystals suspended in varying concentrations of ammonium sulfate show that about 0.2 gm. of ammonium sulfate is associated with 1 gm. of protein irrespective of the concentration of ammonium sulfate in the suspending liquid. In saturated solutions of lithium salts the uptakes of lithium salts by β-lactoglobulin crystals is even greater than of ammonium sulfate and lead to negative values for the 'bound water' or protein hydration. These findings may be interpreted as indicating that the salt ions combine with the protein rather than diffuse into the water of crystallization. Crystal densities calculated from specific volumes are consistent with the idea that salts combine with the protein rather than diffuse into the water of crystallization. The salt uptake of hemoglobin and chymotrypsin-DFP crystals differs from that of β-lactoglobulin in that the uptake of ammonium sulfate varies with the concentration of the suspending medium indicating that these protein crystals have less affinity for ammonium sulfate than does β-lactoglobulin.

Gluconate phosphorylation in yeast extracts. Armand J. Guarino (introduced by H. N. Christensen). *Dept. of Biochemistry and Nutrition, Tufts College Med. School, Boston, Mass.*

Cell-free extracts of bakers' and brewers' yeast contain an enzyme which catalyzes the phosphorylation of gluconate by added adenosine triphosphate. The conversion of acid-labile (ATP) phosphate to acid-stable (ester) phosphate corresponded well with the formation of a substance reactive as 6-phosphogluconate in an enzymatic assay system. The product has also been identified as 6-phosphogluconate by its behavior on paper chromatograms. The enzyme has been purified 8-fold and is free of ATPase, but contains myokinase and some hexokinase. Control experiments show that yeast hexokinase does not catalyze this reaction. The enzyme is magnesium-activated and is optimally active at pH 7.2. Michaelis constants for Mg^{**}, gluconate and ATP are 1.68×10^{-3} M, 1.74×10^{-3} M, and 2.0×10^{-4} M, respectively.

Effect of certain B-complex vitamin deficiencies upon lipogenesis. K. Guggenheim* and R. E. Olson. *Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.*

Thiamin, pantothenic acid, biotin and choline deficiencies were produced in young male rats. Experimental animals with pair-fed and *ad libitum*-fed controls were given 0.1 mM/day/100 gm. body weight radioactive carboxyl labeled acetate mixed in their food over a period of 3 days. At the end of this time the animals were sacrificed, and their liver cholesterol and fatty acids isolated and counted for radioactivity. Although significant variations in total incorporation of radiocarbon into liver lipids in these experiments were found, it was of interest that the specific activity (cpm/mg.) of both fatty acids and cholesterol remained markedly constant in all groups. In the normal *ad libitum*-fed controls about 2% of the total radioactivity given was incorporated into the fatty acids and about 0.3% into cholesterol. In thiamin and pantothenate deficiency only 1% was incorporated into the fatty acids and 0.2% into cholesterol. In biotin deficiency the incorporation into the fatty acids was not significantly different from normal, although incorporation into cholesterol was significantly less. Pair-fed controls behaved like the deficient groups. In choline deficiency, however, 6% was

incorporated into the liver fatty acids. Incorporation into liver cholesterol in these animals was not changed from normal. These results support the view that lipogenesis per se is not specifically altered in these deficiencies.

Imidazole complexes with copper and zinc ions. F. R. N. GURD,* J. T. EDSALL, G. FELSENFELD* AND D. S. GOODMAN.* *Univ. Lab. of Physical Chemistry Related to Medicine and Public Health, Harvard Univ., Cambridge, Mass.*

Imidazole complexes with copper and zinc ions have been studied because of the importance of the imidazole group of the histidine residues in proteins for the binding of metallic ions (GURD AND GOODMAN. *J. Am. Chem. Soc.* In press; also C. TANFORD, *ibid*). The interactions were measured by the changes in pH observed on the addition of cupric nitrate or zinc nitrate to mixtures of imidazolium nitrate and sodium hydroxide in solutions of sodium nitrate (total ionic strength 0.16). The results were analyzed by a method suggested to us by G. Scatchard (compare *Ann. New York Acad. Sc.* 51: 660, 1949). Both cupric and zinc ions were found to bind 4 molecules of imidazole. Tentative consecutive association constants, expressed as log k values, were for copper at 4.5°: 4.58, 3.76, 3.14, 2.18, respectively; and for zinc at 4.5°: 2.76, 2.38, 2.40, 2.22, respectively. The intrinsic logarithmic constants, corrected for statistical factors, are for copper: 3.98, 3.58, 3.32, 2.78, respectively; and for zinc: 2.16, 2.20, 2.58, 2.82, respectively. The magnitudes of the consecutive constants, their dependence on temperature, and the more rapid decrease in the successive values with copper than with zinc are similar to the findings of Bjerrum for the ammonia complexes. The cupric-imidazole complexes show, not only strong absorption in the visible near 6000 Å, but also strong ultraviolet absorption in the neighborhood of 2800 Å; the latter is not observed in complexes of copper with ammonia or amino groups.

Ultraviolet spectroscopy of renin. ERWIN HAAS, HILDEGARD LAMFROM* AND HARRY GOLDBLATT.* *Inst. for Med. Research, Cedars of Lebanon Hosp., Los Angeles, Calif.*

By means of a new isolation procedure, we have succeeded in producing a highly purified preparation of renin. Renin obtained by this procedure has been purified 56,000 times, as indicated by its biological potency. It has not as yet been crystallized and, at the present time, should be considered as a 'renin-protein complex.' This highly active preparation has been investigated by various methods, including ultraviolet spectroscopy, in an attempt to obtain some information about the chemical nature of renin. The results of this study indicate that the renin protein complex can exist in 3 distinct configurations and that, under controlled experimental conditions, a reversible transformation from 1 configuration into the others can be induced. These 3 configurations which will be referred to as the α, β, and γ forms of the renin protein complex, can be distinguished by pronounced differences in their ultraviolet absorption and by differences in their reaction kinetics, such as reaction order and velocity of transformation. The molecular rearrangement, reflected in the formation of the 3 configurations, seems to be a specific property of the renin protein complex which is not shared by other proteins extracted from kidney tissue and which can not be induced in other proteolytic enzymes such as pepsin, trypsin and chymotrypsin. In the α configuration, the ultraviolet spectrum of the renin protein complex coincides with that of other crystallized enzyme proteins. With the formation of the β and γ configuration, the light absorption increases far beyond the range to be expected on the basis of the tyrosine and tryptophan content of the renin protein complex.

Model reactions of phosphorus-containing enzyme inactivators. BRENNIE E. HACKLEY, JR.* AND THEODOR WAGNER-JAUREGG. *Medicinal Chemistry Branch, Chemical Corps Med. Lab., Army Chemical Center, Md.*

In continuation of our studies on the reactions of DFP and related substances with phenols and polyphenols (JANDORF, WAGNER-JAUREGG, O'NEILL AND STOLBERG. *J. Am. Chem. Soc.* In press) we have found, using the Warburg technique for the determination of acid produced, that more complex natural derivatives of catechol, namely *d*-catechin, *l*-epicatechin and caffeic acid have the same reactivity as catechol itself; chlorogenic acid is somewhat more reactive. Gallic acid esters and amides react more rapidly with DFP than does the free acid. Maltol and kojic acid react slowly, pyridoxal and 3-hydroxypyridine more rapidly than does phenol, but less rapidly than catechol. Histidine, carnosine and imidazole (but not 2-methylimidazole, arginine, lysine or proline) accelerate the hydrolysis of DFP without the formation of a stable phosphorylated reaction product. Benzimidazole is only slightly effective. Diisopropylphosphorylated imidazole was obtained by treatment of a chloroform solution of imidazole with the chloro analog of DFP; the reaction product is readily hydrolyzed in water at room temperature, analogous to the formation of imidazole benzoate from 1-benzoylimidazole by exposure to humid air. The possibility of imidazole derivatives being catalysts of phosphorylation by DFP is under investigation. All of the reactions described here are considerably less rapid

than the reaction between DFP and cholinesterase or chymotrypsin. The relation of our findings to current views concerning the nature of the active group in cholinesterase and chymotrypsin will be discussed.

Effects of estradiol on human endometrium and placenta *in vitro*. DWAIN D. HAGERMAN* AND CLAUDE A. VILLEE. *Depts. of Biological Chemistry and Obstetrics, Harvard Med. School, Boston, Mass.*

Tissue slices were prepared of normal human endometrium obtained at hysterectomy performed for reasons other than endometrial pathology and of human placenta obtained at normal parturition. These were incubated in Warburg vessels at 37.5°C. with an oxygen atmosphere in a Ringer-phosphate buffer containing 100 mg.% glucose plus, in some experiments, 10 mM/l. pyruvate. In general agreement with the results of animal experiments, 1–2 μg. of estradiol/ml. incubation medium produced a 9–40% stimulation of oxygen uptake in endometrium; the degree of stimulation varied with the amount of endometrial regrowth subsequent to menstruation. Estradiol increased the Q_{O_2} of term placenta 25% over control values. Estradiol increased glycogenolysis, glycolysis and pyruvate utilization by placenta but its effects on glycogenolysis and glycolysis by endometrium were variable and depended on the period in the menstrual cycle plus other, as yet unknown, factors. At comparable levels, cholesterol had no effect on oxygen uptake, glycogenolysis, glycolysis, or lactate production in placenta. At levels above 4 μg. estradiol/ml. incubation medium there was a decrease in oxygen consumption and marked increases in glycolysis, glycogenolysis and lactate production by endometrium. Similar studies with estriol, estrone and progesterone are underway.

Observations concerning growth factors required by the Donovan Body (*D. granulomatis*). W. KNOWLTON HALL AND ROBERT B. DIENST.* *Depts. of Biochemistry and Microbiology, Med. College of Georgia, Augusta.*

Furhter investigation of the growth factors found in egg yolk, required by the Donovan Body, indicates that the factor or factors are associated with the protein of the yolk. On separation of the yolk the activity was in the lipovitellin fraction. On removal of the lipid from the protein with alcohol-ether the growth promoting activity was retained by the vitellin. Livetin, lipovitellenin and phosphovitin preparations on assay were found to contain variable but considerably lower activity than the vitellin fraction. Digestion of the yolk protein with pepsin or trypsin, or mild hydrolysis with acid or alkali resulted in a loss of growth promoting activity of the protein. The growth promoting factor appears to be a conjugated compound associated with or a part of the protein, which loses its activity on hydrolysis.

Hematological effects of certain 2,4-diaminopyrimidine antagonists of folic acid. LEONARD HAMILTON,* FREDERICK S. PHILIPS,* DONALD A. CLARKE,* STEPHEN S. STERNBERG* AND GEORGE H. HITCHINGS. *Sloan-Kettering Inst. for Cancer Research, New York City and Wellcome Research Labs., Tuckahoe, N. Y.*

Recently 2,4-diaminopyrimidines have been found to be competitive antagonists of folic acid (PGA) for *Lactobacillus casei* (Hitchings *et al. J. Biol. Chem.* 174: 765, 1948). In this respect they resemble 4-amino analogs of PGA. Previous studies have also shown that 4-amino analogs of PGA produce pathological changes in mammals similar morphologically to those associated with PGA deficiency (Philips *et al. Ann. New York Acad. Sci.* 52: 1349, 1950). The 2,4-diaminopyrimidines have shown considerable biological specificity associated with slight changes in fine chemical structure. Among the most active competitive inhibitors of citrovorum factor in *Leuconostoc citrovorum* are the 5-phenylpyrimidines. It was therefore of interest to determine whether a representative of this group acted as a PGA antagonist for hematopoietic tissue. The hematological alterations induced by 2,4-diamino-5-(3′,4′-dichlorophenyl)-6-methyl pyrimidine resemble those caused by 4-amino-PGA in rats and dogs: leucopenia, reticulopenia, hypoplasia of bone marrow in rats, and, in dog marrow, 'megaloblatic' transformation of nucleated erythroid elements with analogous changes in the nuclear chromatin of myeloid cells. Chromosomal aberrations were also frequent in dog marrow. This pyrimidine also produced degenerative changes in the intestinal epithelium of dogs and rats similar to those caused by 4-amino-PGA. Following the administration of 2,4-diamino-5-p-cholorphenyl-6-ethylpyrimidine in dogs identical alterations in the marrow were observed.

Formation of proline from ornithine, citrulline or arginine. PAUL B. HAMILTON. *Dept. of Biochemistry, Alfred I. duPont Inst. of Nemours Fndn., Wilmington, Del.*

Suitable conditions can be selected so that reaction of nitrosyl chloride with ornithine, citrulline or arginine will affect displacement of the α-amino group by a chlorine atom, while the δ-amino, δ-carbamido or δ-guanido groups, respectively, remain relatively intact. α-chloro-δ-amino, α-chloro-δ-carbamido, and α-chloro-δ-guanido-*n*-valeric acid have been prepared in 75, 20 and 75% yields (approximately) from the respective α-amino acid. α-chloro-δ-amino-*n*-valeric acid eliminates HCl in weakly acidic, neutral or alkaline

solution to yield proline. Heated in vacuo to 165° in a sublimation apparatus it yields 3-chloro-2-piperidone (30% which sublimes and proline (70%) which does not sublime. On alkaline hydrolysis, the piperidone ring opens, HCl is eliminated and proline is formed. α-chloro-δ-carbamido-*n*-valeric acid rapidly forms proline, ammonia and CO_2 on alkaline hydrolysis. α-chloro-δ-guanido-*n*-valieric acid is almost quantitatively converted to α-hydroxy-δ-guanido-*n*-valeric (argininic) acid on refluxing with water. In alkaline solution, N-guanyl proline is formed and this breaks down further on alkaline hydrolysis to yield urea and proline. (The author acknowledges with grateful thanks much valuable help from Priscilla J. Ortiz and Roberta J. Anderson.)

Interrelation of thiamine and pyridoxine in *Neurospora*. DANIEL L. HARRIS (introduced by FRANKLIN C. MCLEAN). *Dept. of Physiology, Univ. of Chicago, Chicago, Ill.*

All available pyridoxineless mutants of *Neurospora sitophila* and *N. crassa* grow better at limiting pyridoxine concentrations if thiamine is added to the basal medium. Equal stimulation can be obtained by a mixture of both halves of thiamine. The pyrimidine half alone gives partial stimulation. The thiazole half alone has no activity. Pyridoxine severely inhibits the growth of several mutants requiring both halves of thiamine for optimal growth, when these are the source of thiamine in the basal medium. They are not inhibited if thiamine itself is the source. The inhibition is overcome by the addition of the pyrimidine half, but is increased by addition of the thiazole half. Similar inhibitions are shown by a variety of pyridine compounds resembling B_6, including a number having anti-B_6 activity in Neurospora. These and other data to be presented suggest that pyridoxine and other compounds compete with the pyrimidine for the site on some enzyme, possibly the enzyme which synthesizes thiamin from the pyrimidine and thiazole moieties. They suggest further that pyridoxine may actually be coupled with the thiazole to form an inactive and possibly inhibitory compound. (This work was supported in part by grants from the U. S. Public Health Service and the Wallace C. and Clara M. Abbott Memorial Fund. I am indebted to Merck and Co., Eli Lilly and Co., Lederle Labs., National Drug. Co., Drs. R. M. Mariella and S. M. McElwain for generous gifts of some of the compounds above.)

Reactions of acetyl phosphate catalyzed by 3-phosphoglyceraldehyde dehydrogenase. JANE HARTING* AND SIDNEY VELICK. *Dept. of Biochemistry, Washington Univ., St. Louis, Mo.*

In the presence of inorganic phosphate and DPN, crystalline 3-phosphoglyceraldehyde dehydrogenase from rabbit muscle catalyzes the oxidation of acetaldehyde to acetyl phosphate. (*Federation Proc.* 10: 195, 1951) Four additional reactions of acetyl phosphate and the dehydrogenase have been studied. *1*) The enzyme catalyzes the exchange of the phosphate of acetyl phosphate and radioactive inorganic phosphate. The reaction is slow in the absence of cysteine. No exchange occurs when the DPN bound to the enzyme is removed with charcoal. Although bound DPN is required the exchange rate is many times faster than the oxidation or reduction of DPN. *2*) The dehydrogenase accelerates the disappearance of acetyl phosphate in the presence of arsenate. This arsenolysis is inhibited by inorganic phosphate. *3*) The dehydrogenase can function as a transacetylase. The formation of acetyl-CoA resulting from the incubation of the dehydrogenase, acetyl phosphage, Coenzyme A, and glutathione has been verified by coupling this reaction with two other enzyme systems known to require acetyl-CoA: *a*) the acetylation of sulfanilamide by pigeon liver enzyme, and *b*) the formation of citrate by condensing enzyme and oxalacetate. The rates of the acetylation and the condensation are a function of the Coenzyme A concentration. *4*) Glyceraldehydephosphate dehydrogenase, without added Coenzyme A, can also transfer the acetyl group from acetyl phosphate to glutathione to form a product with the properties of S-acetylglutathione. The four reactions studied are compatible with the theory that an 'acyl-enzyme' complex occurs as the intermediate. The oxidation of acetaldehyde and glyceraldehyde in the absence of added phosphate is consistent wit this mechanism.

Sugar transformations in leaves of *Canna indica*. W. Z. HASSID AND E. W. PUTNAM.* *Div. of Plant Biochemistry, Univ. of California, Berkeley.*

C^{14}-labeled glucose, fructose and sucrose of high specific activity (40 uC/mg.) were infiltrated into discs from leaves of *Canna indica* and the transformation of each of these sugars into other compounds was studied. When C^{14}-glucose was infiltrated, the relative specific activity of this sugar steadily decreased, whereas that of the sucrose increased. No measurable activity could be detected in the free fructose. However, when the sucrose was hydrolyzed to its two monosaccharide constituents, the activity of the fructose was approximately equal to that of the glucose. Similarly infiltration of C^{14}-fructose caused a decrease in the initial high relative specific activity of this sugar and was accompanied by a progressive increase of the specific activity of sucrose. The activity of the free glucose was too low to be determined; but again, the ac-

tivity in the glucose and fructose constituents of the sucrose was approximately equal. The chromatograms indicated the presence of organic phosphates, probably hexosephosphates, which gradually disappeared as time progressed. The fact that no radioactive free fructose could be detected when C^{14}-labeled glucose was infiltrated, but that the glucose and fructose in the sucrose contained equal activity, indicates that the disaccharide is not formed by a mechanism involving the combination of glucose-1-phosphate and free fructose, as in the case of the enzyme form *P. saccharophila*. When C^{14}-labeled sucrose was infiltrated, there was an immediate hydrolysis of the disaccharide, presumably extracellular, as there was no decrease in the sucrose concentration of the tissue.

Further observations on potassium and carbohydrate metabolism. A. BAIRD HASTINGS, CHING-TSENG TENG,* FRANCES B. NESBETT* AND ALBERT E. RENOLD.* *Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.*

Previous observations have shown that normal rat liver slices, incubated in medium (*A*), containing as cations K = 110, Mg = 20, Ca = 10 mM/liter, converted more glucose and pyruvate to glycogen than occurred in a medium (*B*), with K = 40, Na = 70 mM/liter (divalent cations remaining the same). Furthermore, glucose uptake was less in medium (*B*) than in medium (*A*), and glucose formation was greater in medium (*B*) than in medium (*A*). Analysis of the normal liver tissue at the end of incubation in medium (*B*) has shown that the calculated intracellular potassium concentration is about 30% below normal. These changes in carbohydrate metabolism are qualitatively and quantitatively similar to those observed in livers from diabetic animals studied with the same techniques. Experiments have therefore been performed to determine whether insulin, added *in vitro*, would have any effect on carbohydrate metabolism of normal tissue incubated in the moderately low potassium medium (*B*). (Insulin added *in vitro* to normal liver in medium (*A*) had no appreciable effect, whereas its addition to diabetic tissue restored glucose uptake and glucose output to essentially normal values.) The results of our recent experiments on normal livers have shown that under the conditions specified, insulin had the following effects: *a*) glycogen synthesized was increased 50%, *b*) glucose uptake was increased 40%, and *c*) glucose output was decreased slightly, 10%. Comparable experiments are being carried out on livers from adrenalectomized rats with results that are in general opposite to those found for diabetic livers.

Linkage of cystine in proteins. FELIX HAUROWITZ AND MARGARET KENNEDY.* *Dept. of Chemistry, Indiana Univ., Bloomington, Ind.*

In order to evaluate the role of cystine molecules as bridges between the peptide chains of a protein molecule, or as bridges between the subunits of a protein molecule, bovine serum albumin and γ-globulin, edestin and ovalbumin were dissolved in 1.0 N-sodium thioglycolate at pH 8.5. From none of the proteins was cysteine split off. Hence none of them contains the residue —NH·CH(CH_2SSCH_2·$CHNH_2$·COOH)CO— in which one half of the cystine molecule is free. From the intensity of light scattering of solutions of the proteins in thioglycolate and glycolate at pH 8.5, their average molecular weights were calculated. The values found for edestin, γ-globulin and serum albumin in glycolate were approximately 300,000, 200,000 and 100,000 respectively. In thioglycolate the molecular weight of edestin was 300,000, that of γ-globulin 120,000, while the turbidity of serum albumin solutions increased continually owing to the formation of micelles with a weight of several millions. Conclusion: All cystine residues in the examined proteins are bound by both halves of the cystine molecule. The dithio bridges thus formed are essential for the native state of the serum albumin molecules; they link together subunits of the γ-globulin molecule; however, their cleavage in edestin does not change the size of the edestin molecule. (Support of this work by the American Cancer Society upon recommendation of the Committee on Growth, and by the U. S. Public Health Service, is gratefully acknowledged.)

Enzymatic conversion of barbituric acid to urea and malonic acid. OSAMU HAYAISHI (introduced by ARTHUR KORNBERG). *Natl. Insts. of Health, Bethesda, Md.*

The metabolism of uracil and thymine by adaptive enzymes purified from *Mycobacteria* and *Corynebacteria* has been shown to involve oxidation at carbon 4 to yield barbituric acid from uracil and 5-methylbarbituric acid from thymine (HAYAISHI, O. AND A. KORNBERG. *J. Am. Chem. Soc.* 73: 2975, 1951). Studies of the further metabolism of barbituric acid have led to the isolation of an enzyme from uracil-adapted cells which carries out the following reaction: barbituric acid + 2 H_2O → urea + malonic acid. The enzyme was purified from extracts of alumina-ground cells by protamine fractionation and anion-exchange chromatography. Urease in the crude preparations converted the urea to ammonia and CO_2 but this enzyme was absent in the purified preparations. Malonic acid was obtained in crystalline form in 90% of the theoretical yield by evapora-

tion of ether extracts of the acidified incubation mixture and subsequent purification by vacuum sublimation. Melting point, analytic data and qualitative tests established identity with an authentic sample of malonic acid. The 5-methyl-barbituric acid was not attacked by the crude or purified enzyme preparations although it was actively metabolized by thymine-adapted cells.

Enzymatic conversion of desoxycorticosterone to corticosterone. MIKA HAYANO* AND RALPH I. DORFMAN. *Worcester Fndn. for Exptl. Biology, Shrewsbury, Mass.*

The C_{11} hydroxylation of desoxycorticosterone to corticosterone and 17-hydroxy-11-desoxycorticosterone to 17-hydroxycorticosterone have been studied in detail. Beef adrenal homogenate residue obtained at 5000 × *g* will carry on these reactions at such a rate that complete conversion of 5 mg. of starting steroid is obtained in 30 minutes in the presence of tissue equivalent to 5 gm. of fresh gland. The presence of fumarate at 10 times the concentration of steroid and magnesium ions at 5 times and ATP are essential. Malate and succinate are 80 and 50 % as effective as fumarate. All other oxidizable substrates tried were ineffective. Magnesium ions could not be replaced by manganese. The optimum pH of the reaction is around 7.4. Desoxycorticosterone acetate was completely converted to free corticosterone. Desoxycorticosterone glucoside and 21-hydroxypregnenolone also yielded corticosterone at much slower rates. The effect of inhibitors and cofactors on the reaction are now being investigated.

Enzymatic oxidation of bisulfite by rat liver. MURRAY HEIMBERG* AND PHILIP HANDLER. *Dept. of Biochemistry and Nutrition, Duke Univ. School of Medicine, Durham, N. C.*

Washed residue from homogenates of rat liver oxidizes the formaldehyde-bisulfite addition compound (hydroxymethanesulfonic acid) using 1 atom of oxygen/mole with production of formaldehyde and inorganic sulfate. The supernatant from such homogenates catalyzes the same reaction but also oxidizes the formaldehyde further. The optimum pH is about 7.8; all activity is lost after 10 minutes at 100°. The preparations may be kept at 3° for 72 hours with no loss of activity and only partial inactivation is achieved by dialysis against running water or phosphate buffer for 72 hours. The pyruvate-busulfite addition compound is oxidized more rapidly than hydroxymethanesulfonic acid while the analogous compounds formed with acetaldehyde, acetone, *n*-propionaldehyde and *n*-butyraldehyde are not oxidized. Aminomethanesulfonic acid and its N-methyl, N-ethyl, N-n-propyl and N-*n*-butyl derivatives are oxidized at a rate comparable to hydroxymethanesulfonic acid with production of inorganic sulfate, formaldehyde and ammonia or the corresponding amine. Bisulfite in phosphate buffer is rapidly oxidized to sulfate by atmospheric O_2. However, the rate of bisulfite oxidation is markedly slower in the presence of liver residue while virtually no oxidation occurs when bisulfite is incubated with heat-inactivated liver residue. Thus, it appears that oxidation of bisulfite to sulfate in liver is enzymatic. This is the final step in the fate of cysteine sulfur which is known to proceed oxidatively via the sulfenic to the sulfinic acid and then to SO_2 by desulfinase action.

Effect of estrogen on renin-substrate (hypertensinogen). O. M. HELMER AND R. S. GRIFFITH.* *Lilly Lab. for Clin. Research, General Hosp., Indianapolis, Ind.*

As pointed out by Levin (*Endocrinology* 37: 34, 1945) diethylstilbestrol acts as an adrenocorticotrophic substance as judged by several criteria. For example, it markedly lowers the cholesterol content of the adrenal gland. Since ACTH causes an elevation of renin-substrate in the plasma of rats, diethylstilbestrol and other estrogens, and stilbene compounds were administered to rats in order to determine their effect on renin-substrate. The estrogens were much more effective than ACTH in elevating the renin-substrate. As little as 0.02 mg. of diethylstilbestrol/day given subcutaneously for 7 days doubled the renin-substrate content of the plasma of normal rats. In adrenalectomized animals the same dose of diethylstilbestrol caused about one half this elevation of renin-substrate, indicating that its action is in part direct. Twenty compounds have been tested for biological activity by this technique, using normal male rats.

Influence of chorionic gonadotrophin on activity of dehydrogenase in testis which converts 3-hydroxysteroids to 3-ketones. MARY L. HELMREICH, MARY B. LASATER AND LEO T. SAMUELS. *Dept. of Biochemistry, College of Medicine, Univ. of Utah, Salt Lake City.*

It has been previously reported that the interstitial cells of the testis contain an enzyme which will oxidize $\Delta^{5,6}$-3-hydroxy steroids to Δ^{4}-3-ketosteroids. A series of experiments were, therefore, carried out to determine the relationship of this enzyme to the functional activity of the testis and to the action of chorionic gonadotrophin. Rats were maintained on an excellent diet for 2 weeks after hypophysectomy. One group was injected with 100 I.U. chorionic gonadotrophin for 6 days. The testes were then either teased or homogenized, and incubated with pregnenolone and DPN as cofactor under the conditions previously described. Some samples of testes of uninjected rats were pre-incubated for 3 hours with chorionic gonadotrophin; others were in-

cubated with gonadotrophin added at the same time as substrate. Testes of hypophysectomized rats injected with chorionic gonadotrophin, compared with those of uninjected controls, showed 7.4 times the activity per unit weight. The action of the gonadotrophin, however, was not a direct activation of the enzyme system since neither preincubation nor the simultaneous introduction of the hormone increased enzymic activity. It is concluded that the effect was associated with the changes in the interstitial cells which involved time. There was a definite relation between the increase in androgenic activity and the rise in enzymic activity.

Studies on metabolism of iron-containing proteins with radioactive iron and carbon. HAROLD L. HELWIG* AND DAVID M. GREENBERG. *Div. of Biochemistry, Univ. of California School of Medicine, Berkeley.*

A comparative study was made of the uptake of intraperitoneally administered C^{14} of methyl-labeled acetate and radioiron by hemoglobin, plasma protein, myoglobin, liver ferritin, cytochrome-c and bone marrow of guinea pigs. A new method was developed for the isolation of purified myoglobin. In normal animals, the uptake of C^{14} was greatest in ferritin, followed by plasma, marrow, hemoglobin, myoglobin and cytochrome. Incorporation of radioiron was greatest in marrow, followed by plasma, ferritin, hemoglobin, myoglobin and cytochrome. Turnover rates of radioiron and C^{14} in ferritin, plasma and marrow were greater than in the heme proteins. The maxima of C^{14} specific activity in hemoglobin, myoglobin and ferritin occurred approximately 5 days before the maxima of iron specific activity. The half times of the disappearance of C^{14} and radioiron were approximately the same for hemoglobin and myoglobin. Treatment of guinea pigs with polycythemic doses of cobalt for 2 weeks before administration of radioiron increased the uptake of iron by plasma and ferritin. During recovery from polycythemia, the uptake of iron by marrow and hemoglobin was depressed. In the posthemorrhagic state, radioiron was incorporated into hemoglobin and myoglobin at similar rates which exceeded the rate of loss of labeled iron from these proteins over a period of 50 days. The uptake of iron by cytochrome was increased and the turnover of marrow iron was accelerated in bled animals.

Enzymatic studies on ribonucleic acid as measured by streptolysin formation. LEON A. HEPPEL AND R. J. HILMOE.* *Natl. Insts. of Health, Bethesda, Md.*

Yeast ribonucleic acid (RNA) has been shown by Okamoto (*Japan. J. Med. Sc.* 12: 167, 1939) to induce the formation in cultures of *Streptococcus pyogenes* of an hemolysin, streptolysin S, which is apparently a type of nucleic acid. Bernheimer and Rodbart (*J. Exper. Med.* 88: 149, 1948) enriched the RNA effect 100-fold by ribonuclease treatment, alcohol fractionation and dialysis. Their 'active factor' was a nondialyzable polynucleotide. The purpose of the present study is to isolate and characterize this material. A unique opportunity exists here for obtaining a homogeneous polynucleotide by basing the purification upon a quantitative biological effect. It was found that digestion of active factor with intestinal phosphatase up to 50% dephosphorylation caused no loss of activity even though much of the residual-organic phosphorus had been converted to inactive, dialyzable material. With 90% dephosphorylation half of the activity remained. It was all in the nondialyzable fraction which now showed an overall enrichment of 500-fold, based on organic phosphorus. Similar experiments with spleen nuclease showed that active factor could be half converted to dialyzable fragments not precipitable by Mac Fadyen's uranyl-acetate-trichloracetic acid reagent, and again the activity remained, nearly unimpaired, in the nondialyzable residue. The composition of these fractions is under investigation. Various compounds found to be ineffective for streptolysin formation included all four 5'-substituted ribonucleotides, the ribonucleosides and two recently isolated nucleotides kindly supplied by W. E. Cohn, a uridine diphosphate and a cytidine diphosphate.

Inhibition of the growth of *Hemophilus parainfluenzae* by analogues of putrescine. EDWARD J. HERBST, ELEANOR BARTLETT GLINOS AND LAWRENCE H. AMUNDSEN (introduced by E. G. SCHMIDT). *Dept. of Biochemistry, Univ. of Maryland School of Medicine, Baltimore, and Dept. of Chemistry, Univ. of Connecticut, Storrs.*

The diamine, putrescine, is an essential growth factor for *Hemophilus parainfluenzae*, #7901 (HERBST AND SNELL, *J. Biol. Chem.* 181: 47, 1949). Unsymmetrical N-alkyl and N-dialkyl derivatives of putrescine and the corresponding symmetrical putrescine analogues have been tested for *a*) growth factor activity, and *b*) toxicity when added to culture media for *H. parainfluenzae*. All putrescine analogues were completely inactive in promoting growth of the organism, but growth inhibition was generally observed when the analogues were added to putrescine-enriched media. Twenty-two inhibitory alkyl-putrescine derivatives were identified and of this group the highest toxicity was observed in the N-butyl series. The maximum growth response of *H. parainfluenzae* in media containing 2.5 μg. of putrescine/ml. was completely inhibited by 250 μg. of N-*n*-butyl,1,4-

diaminobutane/ml.; total inhibition of growth occurred in the concentration range of 250–1250 μg/ml. of other alkyl-putrescine derivatives. The inhibitors were partially or completely reversed by 25–250 μg. of putrescine/ml. while 1,3-diaminopropane, spermine, or spermidine, which replace putrescine in promoting growth, also antagonized the putrescine analogues. The N-alkyl analogues of putrescine appear to be highly specific antagonists of diamine utilization by *H. parainfluenzae*.

Formaldehyde-pyruvic acid carboligase. HELEN H. HIFT. (introduced by R. MCGILVERY). *Inst. for Enzyme Research, Univ. of Wisconsin, Madison.*

A soluble, stable enzyme has been isolated from rabbit or beef liver which catalyzes an aldol condensation between pyruvate and formaldehyde without requirement for any added factor. The enzyme was extracted from liver mitochondria by a freezing and thawing procedure and was purified some 60-fold by fractionation with ammonium sulfate, and adsorption on calcium phosphate gel. Maximum rates were obtained with formaldehyde in excess, but the stoichiometry of the reaction has been established as involving equimolar quantities of the two reactants. Evidence bearing on the nature and structure of the product of condensation will be presented. Formaldehyde cannot be replaced by any other aldehyde or by formate; whereas pyruvate can be replaced to a limited extent by phenyl pyruvate.

Meat feeding and absorption of labeled calcium. BILLY M. HIGHTOWER AND EDWARD F. WILLIAMS, JR. (introduced by DEMPSIE B. MORRISON). *Dept. of Chemistry, Univ. of Tennessee, Memphis.*

Each of 9 young albino rats was maintained on a diet of 16 gm. of lean meat/day for 5 days. At the end of the 3rd day each rat received by stomach tube a solution of 5 mg. of radioactively labeled calcium as chloride. Seven control rats were maintained on a diet of glucose and Cellufloor for the same period of time. At the end of the 3rd day each of these rats also received by stomach tube 5 mg. of labeled calcium as chloride. Urine and feces were collected for 48 hours after which time the rats were sacrificed and the gastrointestinal tract, the remaining carcass, and blood samples were taken for analysis. It was found that the rats which were maintained on the control diet 'absorbed' an average of 68% of the administered dose of calcium, while the rats which received the meat diet absorbed an average of 46%. The recovered 'nonabsorbed' fraction amounted to 33% for the control group and 48% for the group receiving the meat diet.

The use of ion exchange columns for the isolation of pure amino acids. C. H. W. HIRS (introduced by STANFORD MOORE). *Labs. of the Rockefeller Inst. for Med. Research, New York City.*

Techniques have been developed for the use of 7.5 cm. diameter columns of ion exchange resins for the fractionation of 2–5 gm. of a protein hydrolysate. The separations are performed by elution analysis on 4 columns. A 15-cm. high column of Dowex-50 is used to separate the basic amino acids. A 15-cm. column of IR-4B (XE-59) separates the acidic amino acids, and two Dowex-50 columns (60 cm. and 120 cm.) are used for the neutral amino acids. Elution is effected with ammonium formate or acetate buffers in the pH range 3 to 7. The buffers are removed by sublimation at 40° and the residual amino acids are readily recovered in crystalline form. From 2.5 gm. of bovine serum albumin all the amino acids (methionine and tryptophan excepted) were obtained, analytically pure, in an average yield of 66%. (HIRS, MOORE AND STEIN, *J. Biol. Chem.*, in press). The pure L-antipode was recovered in each case, with the exception of cystine, which was half racemized during the hydrolysis. The alternative procedure of eluting the amino acids from Dowex-50 with 1–4 N HCl is practical when the use of strong HCl is not disadvantageous. The displacement development methods of Partridge and associates have the advantage of much higher capacity; the elution technique is preferable in those cases where its inherently higher resolving power is important.

Application of infrared spectroscopy to the differentiation of trisubstituted steroidal olefines. H. HIRSCHMANN. *Dept. of Medicine, Western Reserve Univ., Cleveland, Ohio.*

Fifty-seven unsaturated and 13 nonethenoid steroids were examined in the region of the out-of-plane bending vibrations of the hydrogen atom attached to a trisubstituted olefine. Δ^5-3β-acetoxysteroids with the usual spatial arrangement of the steroid nucleus and without additional functional groups in the vicinity of the double bond showed in carbon disulfide consistently two bands near 12.31 and 12.50 μ which were absent (or at least much weaker) in their reduction products. Only the position of the lower wave length band was found to vary appreciably with the configuration and nature of the substituent at C-3. Less numerous examples of Δ^7-unsaturated 3β-acetoxysteroids showed maxima near 12.50, 12.06 and 11.82 μ, of Δ^{9-11} compounds a peak between 12.08 and 12.18 μ, and of Δ^{14}-unsaturated steroids 3 maxima near 12.50, 12.37 and 12.14 μ. The introduction of a substituent or double bond at a carbon atom adjacent to the olefinic linkage can profoundly alter the spectrum in the 12 μ region and can reduce absorption intensity well below that of the fairly strong peaks that were seen in this region with certain di-secondary olefines or even with some

nonethenoid steroids. Spectral changes were smaller if the substituted carbon atom was further removed from the double bond but slight shifts could still be detected when these groups were separated by 4 carbon atoms.

Incorporation of N^{15} into nuclear proteins of rat liver. H. D. HOBERMAN AND P. H. PERALTA.* *Dept. of Physiological Chemistry, Yale Univ., New Haven, Conn.*

After injecting N^{15}-glycine into rats, nuclei were isolated from the livers by means of the Behrens' technique. Although the isotope concentrations of nuclei and whole liver were similar, extraction of nuclei with dilute citric acid yielded NPN-free material, the isotope concentration of which was 20% higher than that of the nuclei, while the isotope concentration of a fibrous nucleoprotein prepared by extraction of nuclei with 1 M NaCl and precipitation in 0.14 M NaCl was 60% lower. These fractions accounted for approximately 20% and 5%, respectively, of the N^{15} incorporated in the nuclei, while the remaining N^{15} was approximately equally distributed among 0.14 M-soluble and 0.14 M-, 1 M-insoluble components and the NPN. NaCl fractionation of N^{15}-enriched whole livers also yielded a fibrous nucleoprotein soluble in 1 M NaCl and insoluble in 0.14 M NaCl, the isotope concentration of which was 60% lower than that of the liver homogenate from which it was prepared. This material (Mirsky's 'chromosin') was separated into desoxyribonucleic acid, histone, and residual protein fractions, each of which contained significant amounts of N^{15}. When chromosin was isolated from the livers of groups of rats sacrificed 12, 44 and 60 hours after the last injection of N^{15}-glycine, isotopic analyses demonstrated that the rate of isotope dilution of chromosin and liver homogenate were similar. However, on separating chromosin into the component fractions mentioned above, the dilution of N^{15} was accounted for exclusively in the behavior of the residual protein fraction.

Desoxyribose transfer by nucleosidases of *Escherichia coli*. C. E. HOFFMANN (introduced by J. O. LAMPEN). *Dept. of Microbiology, School of Medicine, Western Reserve Univ., Clevland, Ohio.*

Recent studies by Manson and Lampen (*J. Biol. Chem.* 193: 539, 1951) have shown that extracts of *E. coli* caused the phosphorolysis and arsenolysis of desoxyribosides. In the present experiments, highly dialyzed extracts of *E. coli* B were found to catalyze the arsenolysis of desoxyribose-1-PO_4 and of ribose 1-PO_4. These extracts (essentially phosphate-free) also catalyze the transfer of the desoxyribose moiety (DR-) of thymidine (TDR) to uracil. This transfer was not inhibited by Ba^{++} or arsenate. The formation of reducing sugar from TDR in the presence of arsenate was inhibited by uracil, with the concomitant formation of uracil desoxyriboside. No transfer of DR- of TDR to the purine bases hypoxanthine and guanine occurred without the addition of inorganic phosphate. Transfer of DR- of hypoxanthine desoxyriboside (HXDR) to guanine took place without added phosphate and in the presence of Ba^{++} or arsenate. Transfer of DR- of HXDR to the pyrimidines occurred only in the presence of added phosphate. These studies indicate that the enzymes cleaving desoxyribose nucleosides are DR- transferring enzymes, one type catalyzing the direct transfer of DR- from pyrimidine to pyrimidine and the other from purine to purine. Both types can reversibly transfer the DR- to phosphate and the desoxyribose-1-PO_4 formed is apparently the connecting link in the transfer of DR- between purines and pyrimidines. The phosphate esters are also required for degradation of the desoxyribose chain, since the extracts do not catalyze the degradation of DR- from desoxyribosides unless inorganic phosphate is present.

Effect of Benemid on phosphate excretion in parathyroid tetany. WILLIAM S. HOFFMAN, LUKE PASCALE* AND ALVIN DUBIN.* *Hektoen Inst. for Med. Research, Dept. of Physiology, Univ. of Illinois College of Medicine, and Dept. of Medicine, Stritch School of Medicine, Loyola Univ., Chicago, Ill.*

Benemid, *p*-(di-n-propylsulfamyl)-benzoic acid has been found a powerful agent which reversibly inhibits tubular excretion of penicillin and *p*-aminosalicylate. It also effectively inhibits tubular reabsorption of urate, and is a valuable uricosuric agent in gout. In euparathyroid human and animal subjects, no interference by Benemid of inorganic phosphate reabsorption by tubules has been demonstrated. That this failure may be due to the regulatory action of parathyroid hormone is indicated by studies of Benemid administration in three patients with chronic hypoparathyroidism and tetany. On doses of 2 gm. daily, the serum phosphate concentration fell in 1–3 days to normal or nearly normal levels. This was accompanied by relief of tetanic symptoms. In one patient the serum phosphate level was maintained in the normal range for 4 months with Benemid therapy with complete absence of symptoms. On discontinuance of Benemid administration, serum phosphate rose to pretreatment levels and tetany recurred. Serum calcium levels rose only slowly after weeks of Benemid therapy, showing a dissociation from phosphate levels not seen in parathyroid hormone therapy.

Acid and alkaline phosphatase of degenerating and regenerating peripheral nerve. D. M. HOLLINGER* AND R. J. ROSSITER. *Dept. of Biochemistry, Univ. of Western Ontario, London, Canada.*

The acid and alkaline phosphatase activity (hydrolysis of disodium phenyl phosphate at either pH 4.9 or 9.9) was determined in homogenates of the sciatic nerve of the cat at different times after either nerve section (neurotmesis) or nerve crush (axonotmesis). The intact nerve of the opposite side served as a control. After nerve section the activity of the acid phosphatase, in confirmation of the findings of Bodian (*Symp. Soc. Exper. Biol.* 1: 163, 1947), had increased, reaching a maximum 16 days after the operation. The increase was of the same order (by a factor of 3–4) as the increase in the concentration of desoxypentosenucleic acid (LOGAN, MANNELL AND ROSSITER, *Biochem. J.*, in press). Similar changes were found after nerve crush. The activity of the alkaline phosphatase, on the other hand, decreased rapidly after either nerve section or nerve crush. By 96 days, however, when the crushed nerve was regenerating, the alkaline phosphatase activity of the peripheral segment had increased (by 60% ± 12) in contrast to the usual decrease (by 75% ± 4) found for sectioned nerves when no regeneration was possible.

Enzymatic breakdown of pentose phosphate. B. L. HORECKER AND P. Z. SMYRNIOTIS.* *Natl. Insts. of Health, Bethesda, Md.*

The enzyme in rat liver acetone powder extracts which catalyzes the breakdown of pentose phosphate has been purified 70-fold by a procedure involving fractionation with ammonium sulfate, methanol, and acetone. The purified preparation contains pentose phosphate isomerase which brings about the interconversion of ribose-5-phosphate and ribulose-5-phosphate. Thus either pentose serves as substrate, although the reaction is more rapid with ribulose-5-phosphate. With the purified preparation pentose phosphate disappears very slowly unless an effective system for trapping the reaction products is also added. In the presence of the purified enzyme and crystalline muscle aldolase pentose phosphate is converted to a reducing compound with the properties of a ketoheptose phosphate. This product reacts in the orcinol pentose test; the spectrum which results differs from that given by pentoses but is identical with that obtained with sedoheptulose. In the orcinol spray test (KLEVSTRAND AND NORDAL, *Acta. Chem. Scandinav.* 4: 1320, 1950), (BEVENUE AND WILLIAMS, *Arch. Biochem. Biophys.* 34: 225, 1951) the specific blue color of ketoheptoses is obtained. The dephosphorylated sugar migrates in paper chromatography like sedoheptulose (altroheptulose) but is distinct from glucoheptulose, mannoheptulose, perseulose (galaheptulose) or idoheptulose. Approximately two moles of pentose phosphate disappear for each mole of ketoheptose formed, suggesting combination of pentose phosphate and a two-carbon fragment. The fate of the remainder of the pentose molecule is uncertain; triose phosphate does not accumulate nor is there any appearance of inorganic or alkali-labile phosphate.

Modified $SnCl_2$ reduction method for the determination of serum inorganic phosphate. BENJAMIN N. HORWITT (introduced by A. G. C. WHITE). *Dept. of Medicine, Tulane Univ., Alton Ochsner Med. Fndn., New Orleans, La.*

The sensitive $SnCl_2$ reduction method for the determination of serum inorganic phosphate was investigated. This method was introduced by Kuttner and Cohen (*J. Biol. Chem.* 75: 517, 1927) and modified by Shinowara *et al.* (*J. Biol. Chem.* 142: 921, 1942). Though it was more sensitive than the Fiske-Subbarow method, the color intensity varied with time. Conditions were established which eliminated the above objection, resulted in accuracy favorable to the Fiske-Subbarow method and could easily be run on 0.1 ml. serum with ordinary laboratory equipment. For 0.4 ml. serum it is as follows: the protein free filtrate (PFF) is prepared by adding 0.4 ml. serum to 3.5 ml. H_2O. Add 1 ml. 15% trichloracetic acid and allow to stand 15 minutes. Centrifuge or filter and the supernatant or filtrate is used. 0.5 ml. of the PFF is added to 5.0 ml. of a 10% trichloracetic acid solution. 0.5 ml. of a 1% ammonium molybdate solution is added. Allow to stand 7 minutes. 0.5 ml. of a working $SnCl_2$ solution is added and the blue color is read at 660 mμ after 15 minutes. It is stable over an interval of 10–25 minutes, the time investigated. The working $SnCl_2$ solution is prepared by taking 0.2 ml. of a stock $SnCl_2$ solution (3 gm. $SnCl_2 \cdot 2H_2O$ in 10 ml. conc. HCl) and diluting to 100 ml. with H_2O. this is prepared shortly before use. The amount of phosphate is determined from a standard curve.

Synthesis and metabolic studies of 11-dehydrocorticosterone-21-C^{14} and its acetate. ROBERT SUNG-LAN HSIA,* WILLIAM H. ELLIOTT,* E. A. DOISY, JR. AND SIDNEY A. THAYER. *Depts. of Biochemistry and Internal Medicine, Saint Louis Univ. School of Medicine, Saint Louis, Mo.*

The acetate of radioactive 11-dehydrocorticosterone (Kendall's compound A) was prepared by the diazomethane synthesis from 3,11-diketo-4-bromo-etiocholanic acid chloride and 5 mc. of N-methylurea-methyl-C^{14}. Dehydrobromination was carried out through the semicarbazone by a modification of the Mattox-Kendall method. The activity of the acetate was 1.8×10^6 c/m/mg. The acetate was hydrolyzed by the procedure of Mattox and Kendall to give 11-dehydrocorticosterone-21-C^{14} and then purified by chromatography on a

magnesium silicate-Celite column. Metabolic studies were carried out by intramuscular and intragastric administration of physiological amounts of these cortical steroids. Two hundred μg. of the acetate or of the free compound were given to sparyed adult female rats. The expired air, urine and feces were collected at regular intervals throughout the 4-day experimental period. No appreciable amount of radioactivity was found in the expired carbon dioxide in any of the experiments. Most of the administered radioactivity was recovered in the feces, the major amount being found in the first 24-hour sample.

Cis-trans isomers of vitamin A and retinene in the rhodopsin system. RUTH HUBBARD (introduced by GEORGE WALD). *Biological Labs., Harvard Univ., Cambridge, Mass.*

We have previously shown that rhodopsin is synthesized in solution by a system containing four components: vitamin A, opsin, liver alcohol dehydrogenase, and cozymase. In this reaction, the oxidation of vitamin A to retinene is coupled with the spontaneous condensation of retinene and opsin to form rhodopsin. We have now found that a specific isomer of vitamin A is required for this synthesis. The site of isomer-specificity lies in the condensation of retinene with opsin. Both vitamin A and retinene contain at least two stereochemically effective double bonds. Each can therefore exist in at least four stereoisomeric forms: all-trans, two mono-cis, and one di-cis. All-trans vitamin A or retinene does not form rhodopsin. Isomerization transforms both into effective rhodopsin precursors. We have now isolated a mono-cis isomer of retinene which reacts rapidly and completely with opsin, and yields a rhodopsin which is spectroscopically indistinguishable from the natural product. We have also tested a second mono-cis isomer and a di-cis form and found both to be inactive. A third mono-cis isomer yields a rhodopsin-like pigment with its spectrum displaced about 15 mμ towards shorter wave lengths. (Two of the isomers were crystallized here, and these and two others provided by Distillation Products Industries.) A specific cis-isomer of retinene is therefore required for rhodopsin synthesis. The bleaching of rhodopsin, however, releases *all-trans* retinene. For rod vision to continue, therefore, retinene or vitamin A must be isomerized, or the active cis-isomer of vitamin A must be supplied from the circulation. (This investigation was supported in part by the Medical Sciences Division of the Office of Naval Research.)

Incorporation of radioactive orthophosphate into various nucleotides. F. M. HUENNEKENS, H. R. MAHLER AND E. DIMANT (introduced by HANS NEURATH). *Dept. of Biochemistry, Univ. of Washington, Seattle, and Inst. for Enzyme Research, Univ. of Wisconsin, Madison.*

Previous studies in this laboratory and elsewhere have shown that respiring homogenates of rabbit liver are capable of incorporating P^{32}-labeled orthophosphate into various nucleotide fractions. The present investigation extends this problem through the use of chromatographic methods for the separation of certain of the individual nucleotides. Trichloracetic acid extracts of the homogenate are chromatographed on a column of Florisil (magnesium silicate) and the adsorbed nucleotides are eluted, in order, with: *I*) 2% acetic acid; *II*) water; *III*) 0.5% pyridine, and *IV*) 5% pyridine. The nucleotide fractions, *II*, *III* and *IV*, contain significantly higher amounts of radioactivity in the experimental run than in the blank runs. *Fraction II* is concentrated by barium precipitation and divided into two parts: *IIa* is adsorbed on a Dowex-1 ion-exchange column and eluted according to the procedure of Cohn and Carter. In the eluates, AMP, ADP and ATP are identified by paper chromatography and by light absorption at 260 mμ, while the radioactivities are determined by radioautographs and directing counting; ADP and ATP are labeled. *IIb*, a phenol-soluble subfraction, contains both radioactivity and CoA, the latter being identified by enzymatic assay and paper chromatography. The possible contamination by traces of orthophosphate prevents the unequivocal assignment of the label in CoA. The flavin nucleotides in *fraction IV*, which are isolated by means of partition chromatography between phenol-butanol and water with Celite as the supporting phase, are not labeled.

Factors affecting the synthesis of certain members of the vitamin B-complex in an artificial rumen. CHAS. H. HUNT, O. G. BENTLEY AND T. HERSHBERGER (introduced by A. L. MOXON). *Dept. of Animal Science, Ohio Agricultural Exper. Station, Wooster, Ohio.*

Previous *in vivo* studies (Hunt *et al.*, *J. Nutrition* 25: 207, 1943) have shown that carbohydrates increase the synthesis of riboflavin in the rumen. Through the use of an artificial rumen (Burroughs *et al.*, *J. An. Sc.* 9: 513, 1950) for growing rumen microorganisms, the effect of carbohydrates on the *in vitro* synthesis of 4 B-complex vitamins has been determined. Fistulated steers fed alfalfa hay and a mature, low-protein, timothy hay supplemented with calcium, phosphorus, and trace mineral elements, including cobalt, were used as sources of inocula. The riboflavin, niacin, pantothenic acid, and vitamin B_{12} content of the inoculum was: alfalfa fed steer, 5.8 γ, 25.3 γ, 3.3 γ, and 161.5 mγ/25 ml., respectively; timothy hay fed

steer, 3.9 γ, 18.1 γ, 2.1 γ, and 58.5 γ/25 ml., respectively. Starch additions to the medium used in the artificial rumina increased the production of riboflavin, 126%; niacin, 225%; pantothenic acid, 519%; and vitamin B_{12}, 39% as compared to the amount of vitamins synthesized in control flasks (no added starch). Similar experiments with the timothy hay inoculum showed an increased synthesis with starch of 243% for riboflavin, 191% for niacin, 3135% for pantothenic acid, and 3% for vitamin B_{12}. The limited starch effect on the synthesis of vitamin B_{12} *in vitro* suggests that its elaboration is regulated by nutritional conditions that are different from those for niacin, pantothenic acid, and riboflavin.

Development of ATPase activity in mitochondria by agents and procedures which uncouple phosphorylation. F. Edmund Hunter, Jr. and Sidney Spector.* *Pharmacology Dept., Washington Univ., Saint Louis, Mo.*

Aging, incubation without substrate, lowered tonicity, and treatment with dinitrophenol (DNP), procedures known to cause loss of phosphorylating ability, have recently been shown by several workers to result also in the appearance of ATP splitting activity in mitochondria. Investigation of arsenite, quinacrine, gramicidin, aureomycin, methylene blue, and capryl alcohol indicates that these uncoupling agents also cause appearance of ATPase activity. Treatment of the mitochondria with detergents, butanol, or sonic waves has a similar action. In general the other uncoupling agents are less effective than DNP in this action, some being very much less effective. The appearance of ATPase activity is not the cause of the uncoupling, for it has already been shown that certain phosphorylations carried out by mitochondria are not uncoupled by DNP (*Federation Proc.* 10: 201, 1951). This observation has been extended to several of the other uncoupling agents. The possibility that the uncoupling and the ATPase activity are expressions of a single effect of DNP has been considered. However, under hypertonic conditions, where Potter (*Symposium on Phosphorus Metabolism*, p. 383, 1951) finds no increase of ATPase due to DNP, uncoupling of oxidative phosphorylation appears to occur in the usual fashion. (Supported in part by a USPHS(NIH) grant.)

Studies on the acid-soluble products of the metabolism of orotic acid-6-C^{14}. Robert B. Hurlbert (introduced by Van R. Potter). *McArdle Memorial Lab., Univ. of Wisconsin, Madison.*

Orotic acid-6-C^{14} is incorporated in high yield into rat liver PNA *in vivo* and is catabolized to give $C^{14}O_2$. During early time intervals a large proportion of the C^{14} can be recovered in the acid-soluble fraction of the liver (Hurlbert and Potter, *J. Biol. Chem.*, in press). These radioactive intermediates, at 1 and 2 hours, have been separated by chromatography on Dowex-1 columns (Busch, Hurlbert and Potter, *Federation Proc.* 10: 169, 1951) and on Dowex-50 columns. Two major and three minor peaks of radioactivity were found to include over 75% of the acid-soluble C^{14}. None of the radioactive peaks resembled orotic acid as to chromatographic behavior or UV absorption spectrum. One major peak, eluted from the Dowex-1 columns at 3–4 N HCOOH, was further purified chromatographically and was indicated to be a uridine phosphate, derived from the orotic acid, by the following evidence: The absorption spectrum was that of uridine; the compound contained difficultly hydrolyzable pentose (orcinol-HCl-$FeCl_3$) and 1.01 mole total P/mole uridine, but no labile P (1 N HS_2O_4, 100°, 10 min.); the specific activity (c/min/μM) was 0.06–0.2 times that of the orotic acid; and hydrolysis (8 N HCl, 100°, 1 hour) liberated some radioactive uracil. The other major peak, which was retained more tightly by the Dowex-1 column, had a nearly identical spectrum, pentose content and specific activity, but apparently contained both stable and labile P. Both compounds differed from uridine-3-phosphate in chromatographic behavior and both gave a higher rate of pentose liberation.

Enzymatic phosphorylation of pyridoxal. Jerard Hurwitz (introduced by T. P. Singer). *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

The enzyme which phosphorylates pyridoxal has been purified from brewers' yeast by ammonium sulfate fractionations, dialysis, isoelectric precipitation of contaminating proteins, and alcohol fractionation. This results in a preparation which carries out the reaction: pyridoxal + ATP → pyridoxal-phosphate + ADP. The product, pyridoxalphosphate, was assayed by its ability to activate tyrosine apodecarboxylase prepared from *Streptococcus faecalis R.* The reaction follows O order kinetics and is directly proportional to enzyme concentration. The temperature optimum of the reaction is 48°C, and the pH optimum lies between 6.6 and 6.9. The enzyme requires a divalent metal for activation. Trivalent metals (Al^{+++}, Fe^{+++}) and Ca^{++} inhibit, while Cu^{++} has no effect. Mg, ferrous, Co, Mn, Ni, and Zn ions are capable of activating the system. The enzyme is competitively inhibited by adenine, adenosine, adenylic acid, and inosine triphosphate. Hypoxanthine, inosine, and inosinic acid have no effect on the reaction. The Michaelis constants of the substrates have been determined (pyridoxal = 1.4×10^{-4} M, ATP = 8×10^{-4}). The purified

preparation is still quite heterogeneous. It will not catalyze the phosphorylation of adenosine, ribose, or fructose-6-PO_4, however glucose and riboflavin are phosphorylated. Nevertheless, the enzyme, 'pyridoxal-kinase,' has been shown not to be either of the kinases associated with these substrates.

Kinetic aspects of acetate and lipid metabolism in the intact rat. T. T. HUTCHENS AND J. T. VAN BRUGGEN (introduced by E. S. WEST). *Dept. of Biochemistry, Univ. of Oregon Med. School, Portland.*

Analysis of the time course of blood acetate activity following single intraperitoneal and intravenous doses of C^{14} carboxyl labeled acetate indicates that peritoneal absorption and tissue utilization of the tracer proceed exponentially with half-times of approximately 1 and 7 minutes, respectively. A similar analysis of respiratory CO_2 specific and total activities following $NaHC^{14}O_3$ administration shows that $C^{14}O_2$ clearance from the 'carbonate pool' proceeds with a half time of about 7 minutes. The time course of $C^{14}O_2$ specific and total activities following acetate administration reveals a 20–25 minute half-time component indicating another metabolic compartment between tracer uptake by the cell and $C^{14}O_2$ production. This latter compartment is apparently unshared by the lipogenic processes and its relationship to the citric acid cycle intermediates is being investigated. The above 'kinetic dissection' indicates that acetate metabolism in the intact rat may approximate a multicompartment steady state model. Utilizing the concept of a steady state system and assuming that the 'oxalacetate-acetylating pool' closely approximates the 'lipid precursor pool' one may calculate values for rates of lipid synthesis from data on rate of CO_2 production, R.Q. and the fractions of administered C^{14} incorporated into CO_2, cholesterol and fatty acid at the end of a 4- to 6-hour experiment. That these calculated rates, expressed as half times, agree well with values in the literature tends to confirm the concepts presented. The described technique is useful in quantitating lipogenic rates in short term or chronic metabolic abnormalities.

Interaction of quinacrine with nucleic acids and with their products of depolymerization by alkali and by enzymes. J. LOGAN IRVIN AND ELINOR MOORE IRVIN. *Dept. of Biological Chemistry, Univ. of North Carolina School of Medicine, Chapel Hill.*

Spectrophotometric and partition-equilibrium methods have been used to study the interaction of quinacrine with pentose and desoxypentose nucleic acids and with the products of their alkaline and enzymatic depolymerization. The strength of the binding of quinacrine is a function of the electrical charge on the nucleate anion, which in turn is a function of the size of the nucleic acid molecule. The binding of quinacrine decreases progressively during the course of the depolymerization of the nucleic acids by alkali or by ribonuclease or desoxyribonuclease. This provides the basis for a simple spectrophotometric method for observing such depolymerization and for assaying the enzymatic activity. Even simple nucleotides, such as pure adenylic acids A and B of Waldo Cohn, exhibit appreciable binding of quinacrine. This may aid in explaining the effect of adenylic acid in opposing the inhibition of certain microorganisms by quinacrine. We previously suggested a similar explanation for the analogous, but greater, effect of nucleic acids. The final products of the depolymerization of ribonucleic acid by ribonuclease bind quinacrine more strongly than do the nucleotides produced by the mild alkaline hydrolysis of this nucleic acid. This provides additional evidence that some of the products of ribonuclease action are more complex than simple nucleotides and probably are low molecular weight polymers of the nucleotides as suggested by other investigators.

Abnormal hemoglobins in hemolytic anemias. HARVEY A. ITANO (introduced by CARL G. NIEMANN). *Gates and Crellin Labs. of Chemistry, California Inst. of Technology, Pasadena.*

Three abnormal forms of human hemoglobin have been identified in these Laboratories. Normal hemoglobin, sickle cell hemoglobin and the two unnamed abnormal hemoglobins may be designated adult human hemoglobins *a*, *b*, *c* and *d*, respectively. These species and their mixtures can be distinguished from each other on the basis of their electrophoretic, sickling and solubility behaviors. The hemoglobin mixtures *ab*, *ac*, *ad*, *bc* and *bd* have been found in the erythrocytes of some individuals. The mixtures *bc* and *bd*, which have been found in some individuals with hemolytic anemia, have solubilities which are higher than those of sickle cell anemia hemoglobins but lower than those of sickle cell trait hemoglobins. Our earlier electrophoretic observations have indicated that in most sickle cell anemics only sickle cell hemoglobin is present; more recent electrophoretic investigations employing conditions which yield better resolution of components have disclosed that nearly all specimens of sickle cell anemia hemoglobin contain a small fraction of a component which preliminary experiments indicate to be fetal hemoglobin. Different sickle cell anemia hemoglobin specimens have different solubilities, depending upon the relative amounts of sickle cell hemoglobin and the other components

which are present. The hemoglobins from individuals having both the sickle cell and the thalassemia traits show electrophoretic patterns and solubilities which lie between those observed in sickle cell anemia and sickle cell trait. (This work was supported by a grant from the U. S. Public Health Service.)

Citrus aminopeptidases. EUGENE F. JANSEN, ROSIE JANG* and A. K. BALLS.† *Western Region Research Lab., Bureau of Agricultural and Industrial Chemistry, U. S. Dept. of Agriculture, Albany, Calif.*

A di- and a polyaminopeptidase occur in fairly high concentrations in peel of citrus fruit. The dipeptidase in extracts was destroyed by dialysis against water; however, dialysis of the preparations against dilute sodium oxalate solution resulted in an increase (approximately 1.5-fold) in both activities. The preparations hydrolyzed tyrosine ethyl ester and this activity was not destroyed by water dialysis. Under the conditions tried, diisopropyl fluorophosphate failed to inhibit the peptidases. The peptides alanylglycine, alanylalanine, glycyltryptophane, glycylleucine, leucylglycylglycine and alanylglycylglycine were the most rapidly hydrolyzed. N-acyl derivatives of the peptides were, of course, unhydrolyzable. Esterification of glycylglycine increased the susceptibility of the peptide bond therein to hydrolysis by the citrus preparations. The dehydropeptide, glycyldehydroalanine, was also hydrolyzable. Some but not all peptides containing D aminoacids were hydrolyzable, e.g. the extents of hydrolysis of D,L-alanylglycine, glycyl-D, L-alanine and D,L-alanyl-D,L-alanine were approximately 100, 50 and 75%, respectively. The optimal pH for the hydrolysis of the several peptides was 7.0–7.3. No metal activation of the peptidases was found; however, phosphate inhibited, but only at pH values more alkaline than the optima. The peptidase preparations caused only a slow catalysis of the hydrolysis of proteins; however, peptic digests of proteins were rapidly attacked.

Effects of methionine, ethionine and of certain antibiotics on liver iron. C. A. JOHNSON,* E. R. KIRCH AND O. BERGEIM. *Dept. of Biological Chemistry, College of Medicine, and Dept. of Chemistry, College of Pharmacy, Univ. of Illinois, Chicago.*

Hemachromatosis has been noted in individuals on deficient diets and Kinney, Hegsted and Finch (*J. Exper. Med.* 90: 137, 1949) observed that methionine added to diets of corn grits led to an increase of liver iron in rats. We have studied the effect on young, growing rats and adult rats on adequate casein containing diets of the addition of 3% methionine over periods of 3–7 weeks. In the younger rats on moderate iron intakes methionine increased liver iron significantly. Spleen iron was also increased. Phenylalanine did not have this effect. Ethionine brought about a decrease in liver iron. Vitamin B_{12} led to an increase in spleen but not in liver iron. High doses of neomycin and streptomycin increased liver iron. In adult animals on high iron diets (6% ferric citrate) methionine increased liver and spleen iron. Ethionine alone produced a slight rise in liver iron and a decline in the spleen. Given with methionine it counteracted the effects of methionine on liver and spleen iron. With increases in total iron in the livers the amounts present in HCl extracts as ferrous iron increased. However, the ratios of ferrous iron to total iron markedly decreased. Ratios of ferric to ferrous iron in cecal contents showed no clear relation to liver values. Some alterations were noted in acid extractable sulfhydryl compounds of the liver.

Evidence of a non-nucleic acid, protein bound phosphorus with high metabolic activity. R. M. JOHNSON AND S. ALBERT (introduced by A. H. SMITH. *Richard Cohn Radiobiology Lab., Detroit Inst. of Cancer Research, Detroit, Mich.*

Phosphoprotein isolated from mammalian tissues by the method of Schmidt and Thannhauser is of considerable interest because it incorporates P^{32} at a high rate, second only to that found in acid-soluble fractions. Its existence and particularly its high metabolic activity have been questioned on the ground that the extremely small quantities found may conceivably arise from, or be contaminated by, acid-soluble fractions. After determining the qualitative and quantitative deficiencies of existing methods for obtaining this fraction, modifications were devised to yield more satisfactory preparations. Using these modified techniques studies were made on P^{32} uptake by various phosphorus-containing fractions of nuclei, large and small granules, and cytoplasmic supernatant of rat liver cells. The acid-soluble fractions of large and small granules and supernatant contained more phosphorus and possessed higher specific activities than the phosphoprotein. The reverse was true in the nuclei, where at least 6 times as much phosphorus was found in the phosphoprotein as in the acid-soluble fractions, and the specific activity of the former was at least 4 times as high as that of the latter. It is evident that neither the phosphorus nor the radioactivity found in the phosphoprotein fraction of the nuclei could have arisen as an artifact. Further suggestive evidence for the existence of phosphoprotein as an entity was obtained from *in vitro* studies in which the incorporation of P^{32} into phosphoprotein was found to be coincident with oxidation of tricarboxylic acid cycle intermediates.

Evaluation of hypobromite method for measurement of non-protein nitrogen. FLORENCE L. JONES, MORRIS GIPPIN AND EMANUEL E. MANDEL (introduced by EVANGELINE PAPAGEORGE). *Communicable Disease Center, U. S. Public Health Service, Atlanta, Ga.*

Rappaport's hypobromite method was claimed to permit measurement of NPN with the accuracy of the Kjeldahl method (*J. Lab. Clin. Med.* 32: 1034, 1947). When Rappaport's and Folin-Wu's methods were applied to 88 unselected blood specimens, using routine clinical laboratory technique, agreement between the methods was not satisfactory although no consistent bias appeared to exist statistically. When more exact technique was used and the micro-Kjeldahl method was included in the study, the hypobromite results were consistently lower, constituting 80–89% of the Folin and 78–97% of the Kjeldahl values. Lowest recoveries were given by blood filtrates and urea solutions having the highest nitrogen contents. Diluting filtrates of azotemic blood samples before submitting them to the hypobromite reaction improved the nitrogen yield to within 10% of the Kjeldahl values. This lack of accuracy is attributed to the fact that many NPN components do not react quantitatively with hypobromite. The degree of breakdown not only varies from one component to another but also appears to depend on their concentrations. The hypobromite results therefore are modified by the relative proportions of NPN components present and may vary considerably among specimens with identical Kjeldahl values. The hypobromite method, allowing analysis of micro-quantities and not requiring heat-digestion or a colorimeter, may at times seem more practical than the other two methods studied, but its marked shortcomings must be taken into account.

Oxidation of lactate and pyruvate by thiamine-deficient mice. JAMES H. JONES AND ELIZABETH DE ANGELI.* *Dept. of Biochemistry, Univ. of Pennsylvania School of Veterinary Medicine, Philadelphia.*

Three groups of mice of 6–8 animals per group were made deficient on a diet lacking in thiamine. Each deficient animal had a paired-fed control. When markedly deficient both animals of each pair of *group 1* were given, by stomach tube, an aqueous solution containing 37 mg. of carboxyl C^{14} sodium lactate (2700 counts/mg. carbon) CO_2 was collected in hourly intervals for 3 hours, radioactivity in c/min/mg. C were determined and total counts/minute calculated (counts/mg. carbon multiplied by mg. of CO_2 carbon). Animals of *group 2* were given 37 mg. of α,β C^{14} sodium lactate (2400 c/min/mg. C) and those of *group 3* 37 mg. of α C^{14} sodium pyruvate (3100 c/min/mg. C), and the CO_2 collected and the activity determined as above. All data were treated statistically. In respect to counts/minute/mg. of carbon there was no difference between deficient and control animals. In some hourly collections the mean of the deficient animals was slightly more than that of the controls, and in other hourly collections the reverse was true. In no case was the difference between the means significant ($P = 0.05$). In the total counts/minute the means of the control animals were, without exception, larger than the corresponding means from the deficient animals. At times the difference between means of deficient and control groups were rather large, but due to the wide variation of individual values, in one hourly collection only was the difference between the means significant. All counts were calculated to infinite thickness.

Non-enzymatic oxidation of α-ketoglutarate. GEORGE KALNITSKY. *Dept. of Biochemistry, State Univ. of Iowa, Iowa City, Iowa.*

α-Ketoglutarate (α-KG) can be oxidatively decarboxylated to succinate and CO_2 in the presence of the specific enzyme and co-factors (Mg^{++} or Mn^{++}, adenosine triphosphate and diphosphothiamine). The oxidation of α-KG can also be accomplished with Mn^{++} in veronal buffer *with no enzyme present*. Mg^{++} is inactive. This non-enzymatic oxidation is accelerated by increasing concentrations of 1) Mn^{++} or Co^{++} 0.01–0.03 M), 2) α-KG (0.01–0.04 M), and (3) O_2. The α-KG was identified by melting point and mixed melting point determinations and by the absorption spectrum of its 2,4-dinitrophenylhydrazone. With veronal buffer, the optimum pH for this non-enzymatic reaction is 7.9–8.1. Inorganic phosphate and citrate buffers strongly inhibit. One mole of O_2 is taken up and 0.7 mole of CO_2 is liberated/mole of α-KG utilized. Very small amounts of succinate are formed. The other products of this reaction have not yet been identified. Glycine (10 μM/2.0 ml. total volume) and other amino acids accelerate the speed of the reaction. α-Amino acids (α-amino butyric acid) are more effective than the corresponding β- and γ-amino acids. *Hydroxy* amino acids (threonine, serine, hydroxyproline) are more active than the corresponding amino acids (α-amino butyric, alanine, proline). The most rapid rates of oxidation of α-KG are obtained with threonine, serine and lysine, the slowest rates with cysteine and histidine. Peptides (diglycine and triglycine) are not as effective as glycine.

Preparation of suspensions of intact liver cells. JOHN PAUL KALTENBACH (introduced by G. A. LEPAGE). *McArdle Memorial Lab., Univ. of Wisconsin, Madison.*

A method was devised to obtain suspensions of intact cells from liver in order to compare the metabolism of isolated whole cells with that of tissue homogenates, slices and ascites tumor cells. Suspensions consisting mainly of intact cells were obtained by gently forcing partially decapsulated liver through a 20-mesh stainless steel screen. The expressed material was suspended in 8–10 volumes of suitable medium, passed through a 60-mesh screen, and then washed by repeated low-speed centrifugations and resuspensions. The yield of intact hepatic cells from rats and mice was approximately 5–10%. Hepatic cells obtained from small animals tend to be fragile resulting in a low yield of cells. Examination of the cell suspensions by phase microscopy, as well as studies comparing rates of anaerobic glycolysis with and without hexosediphosphate present, was used to evaluate the integrity of the cell membrane in these preparations. Uptake of glycine-2-C^{14} into the proteins of the cell suspensions was approximately tenfold higher than in homogenates fortified with cofactors (LEPAGE, *Cancer Res.*, in press).

Pyridine nucleotide transhydrogenase. NATHAN O. KAPLAN, SIDNEY P. COLOWICK AND ELIZABETH F. NEUFELD.* *McCollum-Pratt Inst., Johns Hopkins Univ., Baltimore, Md.*

A purified preparation of pyridine nucleotide transhydrogenase from *Pseudomonas fluorescens* (COLOWICK *et. al.*, *Federation Proc.* 10: 174, 1951) has been found to catalyze each of the following reactions: *1*) $TPNH_2 + DPN \rightarrow TPN + DPMH_2$; *2*) $TPNH_2$ + desamino DPN → TPN + desamino $DPNH_2$; *3*) desamino $TPNH_2$ + DPN + desamino TPN + $DPNH_2$; *4*) $DPNH_2$ + desamino DPN ⇆ DPN + desamino $DPNH_2$; *5*) $TPNH_2$ + desamino TPN → TPN + desamino $TPNH_2$. Whereas *reaction 1* and the analogous *reactions 2* and *3* do not proceed readily in the reverse direction, *reaction 4* is readily reversible. The reversibility of *reaction 5*, which, like *reaction 4*, resembles an exchange reaction, is under investigation. Some evidence has now been found for the reversibility of *reaction 1*, by coupling the transhydrogenase system with the TPN-specific cytochrome *c* reductase from liver or with the TPN-specific glutathione reductase from peas. TPN and inorganic phosphate concentrations must be kept low, since these substances inhibit reaction 1 in both directions. Transhydrogenase activity has been found in extracts of *Pseudomonas aeruginosa* and in several strains of *Azotobacter*. J. Stern's observation (unpublished) of the presence of the enzyme in rat heart has been confirmed. The significance of the transhydrogenase reaction in electron transport will be discussed.

Absorption of vitamin A ester and alcohol in conditions of impaired fat absorption and digestion. MARGARET M. KASER, RICHARD MINTON AND CLARA V. HUSSEY (introduced by ARMAND J. QUICK). *Veterans Admin. Center, Wood, Wis., Marquette Univ., Milwaukee, Wis.*

Serum levels of vitamin A in human subjects were determined in blood samples drawn before the oral administration of 200,000 IU of vitamin A ester and 3, 5 and 8 hours later. About a week later the procedure was repeated with vitamin A alcohol. In 2 patients with a sprue-like syndrome no increase in serum vitamin A was obtained with either form of vitamin A. However, 6 patients with chronic pancreatitis, in 2 of whom a deficiency of pancreatic lipase was determined by analysis of pancreatic juice, showed no response to vitamin A ester, but had increases of serum vitamin A following administration of the alcohol of 170 to 1249 IU/100 ml. Three normal dogs given 3000 IU/kg. of body weight showed similar curves with both forms of vitamin A with increases of 175 to 766 IU/100 ml. of serum. Two animals after cholecystonephrostomy absorbed both forms poorly. The maximum increase of serum vitamin A was only 109 IU/100 ml. This defect in absorption was not corrected by the administration of desiccated bile.

Chromatographic separation of adrenal cortical hormones and related steroids. E. R. KATZENELLENBOGEN,* T. H. KRITCHEVSKY* AND KONRAD DOBRINER. *Sloan-Kettering Inst. for Cancer Research, New York City.*

A chromatographic system has been developed which permits ready separation of adrenocortical hormones such as the acetates of 17-hydroxycorticosterone, cortisone, corticosterone, 11-dehydrocorticosterone and Reichstein's compound S. The separation of less polar compounds such as androsterone and etiocholanolone can be similarly achieved. The 'partition' column used is silica gel partially saturated with ethanol or methanol and the developing solvent is methylene chloride containing either alcohol. The polarity of the eluting solvents may be adjusted over a wide range by variation of the alcohol content as well as by the admixture of petroleum ether. The separation of metabolites of the adrenal cortical hormones and the use of the method for the isolation of steroids from biological materials will be described.

Choline oxidase system. BARBARA KELLEY (introduced by JOHN R. TOTTER). *Dept. of Biochemistry, Univ. of Arkansas School of Medicine, Little Rock.*

It has been found that riboflavin added at a level of 15 mg/kg. of diet brings about an elevation of the choline oxidase activity in the livers of rats fed various purified diets. This would suggest that flavin enzymes might be involved in the

choline oxidase system. Atabrine, which has previously been shown to inhibit flavin enzyme systems, was tested *in vitro* and *in vivo* for its effect on the choline oxidase system. *In vitro* atabrine was found to bring about an inhibition of choline oxidase activity as measured by the Warburg manometric technique. This *in vitro* inhibition could not be overcome by the addition of FAD, riboflavin, ATP, citrovorum factor, pyridoxine or by various combinations of these possible co-enzymes. The supernate from heated kidney, as a source of co-enzyme, was also ineffective. *In vivo* riboflavin has also been shown to elevate the liver choline oxidase activity of atabrine fed rats as measured both spectrophotometrically and manometrically. The effect of feeding folic acid will also be discussed.

Determination of fructose in presence of glucose in blood and urine by use of diphenylamine. A. B. KENDRICK (introduced by MARTIN E. HANKE). *Dept. of Medicine, Univ. of Illinois, Chicago.*

Fructose reacts with diphenylamine much faster than glucose. The reagent is prepared by dissolving 3 gm. diphenylamine in 110.4 ml. glacial acetic followed 13.2 ml. water and 36.4 ml. conc. HCl. This 10% reagent is more suitable than the 16.4% HCl reagent used by Harrison for the determination of inulin. The extinction coefficients per millimol with 635 filter and Evelyn colorimeter were 6.63 for fructose and 0.21 for glucose. Blood and urine filtrates were prepared in 1–80 dilutions, using $CdSO_4$ and NaOH of Byfield (Masters Dissertation, 1943). Five-ml. samples of the filtrates were mixed with 10-ml. portions of the diphenylamine reagent and heated for *exactly* 15 minutes in a *boiling* water bath. After cooling, the blue color was measured. A 3% correction, found for glucose, is applied to the reducing sugars other than fructose: fructose = apparent fructose − 0.03 (total sugar − apparent fructose). When glucose concentration is less than 200 mg. %, the glucose correction may be avoided by decreasing the reaction time to 10 minutes. A sample of normal fasting blood contained 83 mg. glucose and 0.5 mg. fructose. To this blood was added 400 mg. glucose and varying amounts of fructose from 20–200 mg.%. Quantitative recoveries of fructose were found with a maximum error of 5%. An average value for glucose and 'fructose' in 12 samples of urine voided just before breakfast was 65 and 19 mg., respectively.

Synthesis of phospholipids in isolated mitochondria. EUGENE P. KENNEDY (introduced by ALBERT L. LEHNINGER). *Ben May Lab. for Cancer Research and Dept. of Biochem., Univ. of Chicago, Chicago, Ill.*

The synthesis of phospholipids in isolated, washed mitochondria from sucrose homogenate of rat liver has been studied with the aid of radioactive phosphate. In confirmation of the data of Friedkin and Lehninger (*J. Biol. Chem.* 177: 775, 1949) this process has been found to be dependent upon oxidative phosphorylation, and severely inhibited by the addition of 2,4-dinitrophenol. Synthesis of radioactive phospholipid by washed mitochondria is approximately doubled by the addition of glycerol, which suggests that the incorporation of P^{32} is not due to a simple replacement reaction, but rather is indicative of a complete synthesis of the phospholipid structure. Evidence confirming this concept has been obtained from experiments with C^{14}-labeled myristic acid, which show that fatty acids are also incorporated into the phospholipid molecule by isolated mitochondria. Isotope dilution experiments with unlabeled glycerophosphate reveal that inorganic P^{32} is incorporated into the phospholipid by a pathway which involved glycerophosphate, or a compound in close equilibrium with glycerophosphate. Details of these experiments will be discussed.

Isotopic derivative method involving unfractionated mixtures of labelled compounds as indicators: amino acid estimations. ALBERT KESTON AND JOSEPH LASPALLUTO.* *New York Univ. Coll. of Medicine, New York City.*

Quantitative derivitization is unnecessary in the estimation of organic compounds as isotopic derivatives if the compound of interest labelled with another isotope is added before reaction with the radioactive reagent (*Federation Proc.*, 10: 207, 1951). This apparently required availability of each compound analyzed in pure and isotopic form. However the method has been developed so that crude mixtures of labelled compounds may be used as indicators for analyzing amino acid mixtures and protein hydrolysates. Mixtures of isotopic amino acids with desired optical configurations are easily prepared biosynthetically. Major difficulties of syntheses and resolutions previously encountered in isotope derivative and dilution methods are avoided for both methods by using labelled protein hydrolysates as indicators or diluents. We have used as indicators, protein hydrolysates prepared from Thiobacillus thioparus raised on $C^{14}O_2$ as sole carbon source. Amounts of particular C^{14} amino acids are found by diluting the labelled hydrolysate with known amounts of unlabelled compounds, reacting the undiluted and the diluted hydrolysate separately with I^{131} pipsyl chloride, and comparing the $C^{14}I^{131}$ ratios of the pipsyl derivatives of the compounds of interest isolated from both reaction mixtures. The analyzed C^{14} labelled protein hy-

drolysate may be used as indicators for the analysis of amino acid mixtures, adding definite amounts of the labelled hydrolysate before reaction with pipsyl chloride and comparing ratios of the two isotopes in isolated samples as described in *Federation Proc.*, 10: 207, 1951. Beta lactoglobulin analyzed by this method gave the following values: glutamic acid, 18.3%, aspartic acid 10.4%, serine, 3.90%, threonine, 5.00% and glycine, 1.52%.

Isolation of globulins of cellular nuclei. WILLIAM R. KIRKHAM (introduced by LLOYD E. THOMAS). *Dept. of Biochemistry, Univ. of Missouri, Columbia.*

Nuclei of calf thymus and calf liver were isolated by a modified Behrens method and then extracted with a 0.14 M sodium chloride solution. When the extracted proteins were subjected to electrophoresis, the results indicated, in each case, the presence of one principal component and a few few components present in much smaller quantities. The principle component was precipitated by dialyzing the solution against water and adjusting to pH 5.5. The total protein extracted by 0.14 M sodium chloride constitutes one third to one half of the total dry weight of the nuclei. The solubility of the principle component in salt solutions places it in the globulin class. Other characteristics are described.

Decrease in oxygen uptake of duck erythrocytes following whole-body x-irradiation.* J. RAYMOND KLEIN. *Biology Dept., Brookhaven Natl. Lab., Upton, L. I., N. Y.*

Decrease in the rate of oxygen uptake of erythrocytes from the Pekin duck follows whole-body x-irradiation (250 KV, 30 ma, HVL 2.8 mm. Cu). The decrease, evident 1–2 days after irradiation and maximum after 3–4 days, is clearly appreciable with 500 and maximum with 1500 r. The addition of methyl ene blue to the cells, while producing a higher rate of uptake, does not prevent the depression following irradiation. This suggests the deficit induced by irradiation is below the level of the cytochromes. Interchange of plasma between blood from control and irradiated animals has no effect on the uptake of the cells indicating neither lack in the plasma of the irradiated animals nor presence of toxic material. Irradiation of erythrocytes *in vitro* with doses the same or higher than given the whole animal has no effect on the oxygen uptake either immediately or after 24 hours. Thus, the decrease in uptake of the erythrocytes following irradiation of the whole animal appears not to involve simple, stoichiometric inactivation by x-rays of enzymes participating in the respiration of the cells. (Research carried out under the auspices of the U. S. Atomic Energy Commission.)

Binding of neutral organic molecules by proteins. IRVING M. KLOTZ AND JANET AYERS. *Dept. of Chemistry, Northwestern Univ., Evanston, Ill.*

To complement earlier investigations of the interactions of proteins with organic ions (cationic and anionic), the binding of uncharged aminoazobenzene has also been studied. Complex formation with bovine and with human serum albumin becomes significant as the pH of the solution approaches 7 and continues to increase to pH 9. Free energies of formation of the first albumin-dye complex are approximately −6 and −7 kcal/mole, respectively, at the pH's cited. At pH 9, the binding of the neutral molecule is comparable to that of methyl orange, an anion of roughly equal size. Similarly, the increase in uptake of aminoazobenzene with increase in pH parallels the behavior of methyl orange, although the rate of increase is greater with the neutral molecule. Previous evidence of changes in the configuration of the albumin molecule with variations in pH are thus confirmed. The stability of aminoazobenzene complexes with albumin, unlike those of methyl orange, is markedly sensitive to temperature, the heat of the reaction being nearly −4 kcal/mole and the entropy change about 10 EU. The magnitudes of these thermodynamic quantities are consistent with the fact that a neutral molecule is being bound to the protein. Like methyl orange, aminoazobenzene is not bound by bovine γ-globulin at pH 7. Furthermore binding of either small molecule by albumin is reduced in the iodinated protein. Evidently at least some sites on the protein molecule have an affinity for both neutral and negatively-charged organic molecules.

Fe^{+++} — Fe^{++} change in coupled oxidation reaction of tryptophan peroxidase. W. E. KNOX. *Harvard Med. School, Boston, Mass.*

The liver tryptophan peroxidase catalyzes the physiological oxidation of L-tryptophan to formylkynurenine. Two unusual properties appeared to set this enzyme apart from the classical peroxidases for which no biological functions have yet been found: *1*) the absolute specificity for its natural substrate; *2*) its function only as a coupled oxidation system, with the peroxide needed being generated in the same reaction by a second oxidative step. The actions of horse radish, milk and white cell peroxidases and the peroxidase-like action of methemoglobin have been compared with that of the tryptophan peroxidase in order to elucidate the basis for the above properties and to determine if the other peroxidases might possess similar properties also fitting them for biological functions. All the enzymes were tested for their ability to act in a catalase-containing coupled-oxidation system with peroxide from glucose

oxidase. Their specificity on a series of substrates and their sensitivity to various inhibitors were also determined. All the enzymes were active with enzyme generated H_2O_2. Milk and white cell peroxidases were especially active due to the absence of the usual inhibition of them by bottle-H_2O_2. Each of the peroxidases, but not methemoglobin, showed evidence of an individual type of substrate specificity. None approached the absolute specificity of the tryptophan peroxidase. All the enzymes were inhibited by the usual reagents for Fe^{+++}, but only the tryptophan peroxidase was also inhibited by 0.001 M. Cu^{++} and by CO. CO inhibition was partially reversed by light. The tryptophan peroxidase therefore appears to differ from the other peroxidases by undergoing a cyclic $Fe^{+++} - Fe^{++}$ change, in the process of catalyzing the two successive steps of its coupled oxidation.

Origin of phage purines. ARTHUR L. KOCH (introduced by E. A. EVANS, JR.). *Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.*

Escherichia coli was grown in a medium containing as sole nitrogen source: *a*) 750 mg. of 62 atom % excess $N^{15}H_4Cl$, and *b*) 201 mg. of 8-C^{14}-adenine containing no excess N^{15} and counting 8×10^3 c.p.m./μM as an infinitely thin sample. An aliquot of bacteria was partitioned and the bases isolated by chromatography on anion exchange columns and then purified on cation columns. Samples of the 2 purines were deaminated. Little N^{15} was found in the nuclear nitrogens of the purines, and the specific radioactivity of the purines was equal to that of the medium adenine. This indicates that all purines had been derived almost completely from the medium adenine. Other nitrogenous constituents of the cell contained 50 to 60 atom per cent excess N^{15}. The remaining bacteria were infected with phage T_6r+ in an unlabeled medium containing no purines, and the progeny were similarly analyzed. From a comparison of the radioactivities of host and phage purines, it appeared that 65–75% of the purines of the phage were synthesized after the cells had been infected. However, the nuclear nitrogens of the phage purines contained little N^{15}. It is concluded that the new synthesis of purine nitrogen occurs entirely from the ammonium ion of the medium rather than from any nitrogenous constituent of the bacterial cell.

Free cholesterol ester relationship as determined by digitonin precipitation and low temperature solubility separation. ALFRED E. KOEHLER AND ELSIE HILL.* *Sansum Clinic Research Fndn. and Santa Barbara Cottage Hosp., Santa Barbara, Calif.*

Free serum cholesterol can be separated from the esters by solubility at −60° using 3 mg. total cholesterol in 10 cc. methanol. The values for this method agree with the digitonin separation within the limits of reproducibility of the determination of cholesterol. For 18 normal subjects the difference of the ester percentage of the total was 1 ± 2.1 lower for the solubility method and in 23 subjects with various pathological conditions including hepatic cirrhosis and arteriosclerosis the ester percentage of the total was 3 ± 3.6 higher. Further fractionation of the esters by solubility of 3 mg. ester in 10 cc. ethanol at −60° showed a fair degree of uniformity in ester composition in normal subjects but showed considerable variation in the abnormal group. In hepatic cirrhosis particularly the esters were of the more insoluble type.

Cholesterol serum protein relationship. ALFRED E. KOEHLER AND ELSIE HILL.* *Sansum Clinic Research Fndn. and Santa Barbara Cottage Hosp., Santa Barbara, Calif.*

In shaking 2 cc. serum from 18 normal subjects with 10 cc. benzene at 25° for 15 hours, only 3–7% of the total cholesterol was extracted. In 31 subjects with various disease conditions including 9 with severe arteriosclerotic heart disease the variation was 2–8% with approximately the same average. The only deviation was in nephrosis where in 4 subjects the amount extracted ranged from 33–72%. However, by means of a high speed (10,000 RPM) rotary extractor all of the cholesterol can be extracted in 5 minutes at 20° or 15 minutes at 25° with benzene, ether or chloroform. Denaturization of the serum proteins by various means does not increase the extractability by shaking of cholesterol with benzene or chloroform. The rate of extraction at 3,000 rpm at 10° show a uniformly progressive curve for the various serum lipide fractions. The amount extracted is a function of the unextracted portion. The order of the rate of extraction as expressed by per cent of their respective totals is free cholesterol, ester cholesterol, phospholipides and fats. We have found no significant alteration in the rate of extraction in various pathological conditions including arteriosclerosis. These experiments indicate that there is no basis for the belief that there is a lipide protein combination. The relationship is apparently purely one of adsorption.

Conversion of epitestosterone to Δ^4-androstene-3,17-dione by rabbit tissues. CHARLES D. KOCHAKIAN, DAN M. NALL* AND NICHOLAS PARENTE.* *Oklahoma Med. Research Inst. and Hosp., Oklahoma City, Okla., and Univ. of Rochester, Rochester, N. Y.*

Testosterone has been shown to be metabolized by rabbit liver slices and liver and kidney homogenate to Δ^4-androstene-3,17-dione and epitestosterone. Δ^4-Androstene-3,17-dione in turn is metabolized by rabbit liver slices to testosterone and

epitestosterone. It has been demonstrated now that epitestosterone is converted to Δ^4-androstene-3,17-dione by rabbit liver slices and liver and kidney homogenates. The amount of conversion was similar (approximately 0.5%) in the 3 experiments. As much as 96% of the added epitestosterone was recovered. (This investigation was supported by the American Cancer Society on recommendation of the Committee on Growth of the National Research Council.)

Isotopic studies on reaction mechanism and equilibrium in enzymatic decarboxylation of amino acids. RAY KOPPELMAN,* STANLEY MANDELES* AND MARTIN E. HANKE. *Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.*

Lysine and glutamate were enzymatically decarboxylated in the presence of 99.8% D_2O. The corresponding amines (cadaverine and gamma amino-butyrate which were formed) contain one and not more than one deuterium atom located on what was the alpha carbon of the amino acid. Any reaction mechanism which postulates an imino acid before decarboxylation and consequent exchange of two deuterium atoms is therefore excluded. Lysine was exhaustively decarboxylated enzymatically in the presence of $C^{14}O_2$. The minute equilibrium concentration of lysine was determined by addition of a measured amount of inactive carrier lysine and by estimation of the radioactivity of the CO_2 formed by the decarboxylation of the lysine mixture. The equilibrium quotient (amine) (HCO_3^-)/(amino acid), was 500 when the initial reactant was the amino acid, and 1000 when the initial reactants were the amine plus CO_2. These quotients were maintained throughout three-fold changes in the effective opportunity for enzyme action. Our interpretation postulates that the enzymatic decarboxylation involves at least three reversible steps: *1*) formation of an enzyme-amino acid complex (probably a Schiff base between the amino acid and pyridoxal phosphate, the coenzyme for these decarboxylations); *2*) loss of CO_2 from this complex to form an amine complex; and *3*) subsequent dissociation of the amine complex to form the amine plus the enzyme. The difference in the equilibrium quotients indicates that the decarboxylation is distinct from and much more rapid than the formation of the amine.

Studies on the enzymatic synthesis of phospholipides. ARTHUR KORNBERG AND W. E. PRICER, JR.* *Nat'l. Insts. of Health, Bethesda, Md.*

The only demonstration of phospholipide synthesis in cell-free systems is the inclusion into a phospholipide fraction of labeled inorganic phosphate and choline by tissue homogenates (B. A. FRIES, H. SCHACHNER, AND I. L. CHAIKOFF, *J. Biol. Chem.* 144: 59, 1942; M. FRIEDKIN AND A. L. LEHNINGER, *J. Biol. Chem.* 177: 775, 1949; C. ARTOM, M. CROWDER, AND M. A. SWANSON, *Federation Proc.* 10: 157, 1951). The present report describes the incorporation into phospholipide fractions of more complex labeled precursors by the action of partially purified enzyme preparations. Aqueous extracts of rat liver fractionated with methanol at low temperature convert α-glycerophosphate (α-GP^{32}) into a derivative(s) which is soluble in alcohol, acetone and ethyl ether but is insoluble in water. The rate of formation of this derivative is increased 3-fold by coenzyme A (50% pure), 2–3 fold by stearate, palmitate, oleate and other long-chain fatty acids and 40-fold by ATP. In the presence of these factors and of enzyme equivalent to 1 gm. of rat liver, 1 μ mole of α-GP^{32} (10% of the original addition) is converted in 30 minutes at 22°. The α-GP^{32} derivative has been partially purified by paper and anion-exchange chromatography but has not been definitely characterized. Phosphorylcholine, labeled with P^{32} and/or C^{14} (in C_1 and C_2) is converted by similar liver preparations into a compound(s) insoluble in water but soluble in lipide solvents. The rate of conversion of C^{14} phosphorylcholine is approximately 10 times that of C^{14} choline. With doubly-labeled phosphorylcholine (P^{32}, C^{14}) the ratio of P^{32} to C^{14} in the product approximates that in the substrate, suggesting the incorporation of phosphorylcholine as a unit into a phospholipide molecule.

Retention of configuration and the mechanism of enzyme action. DANIEL E. KOSHLAND, JR. (introduced by D. D. VAN SLYKE). *Biology Dept., Brookhaven Natl. Lab., Upton, N. Y.*

In the enzymatic reactions catalyzed by most of the phosphorylases, carbohydrases, and phosphatases, a group, *X*, at an asymmetric carbon atom is replaced by a group, *Y*, without change in the configuration of the carbon atom. This is an apparent contrast to analogous nonenzymatic reactions in which a single step replacement is accompanied by racemization or inversion. The anomaly is resolved by postulating two general types of mechanism to explain the enzymatic retention. *a*) Replacement does not actually involve cleavage of the C-X bond. For example, if X is an OR group, hydrolysis would proceed with breaking of the O-R rather than the C-O bond. *b*) Replacement occurs with cleavage of the C-X bond but proceeds in two steps, each involving an inversion. In the first step, an electron-sharing site on the enzyme surface makes a 'backside attack' on the asymmetric carbon atom, leading to an enzyme-substrate intermediate with inverted configuration. In the second step, *group Y* displaces the enzyme and forms a product with the

same configuration as the initial substrate. The strength of the C-enzyme bond in the intermediate may vary from a full covalent bond to a weak solvate-type interaction, but the total enzyme-substrate interaction must be sufficient to prevent racemization. Evidence from isotopic experiments, specificity studies, and the mechanism of the non-enzymatic reactions is presented in support of these explanations. (Research carried out at Brookhaven National Laboratory under the auspices of the U. S. Atomic Energy Commission.)

Instability of reduced DPN ($DPNH_2$) in the presence of glyceraldehyde-3-phosphate dehydrogenase. EDWIN G. KREBS AND GALE W. RAFTER (introduced by EARL R. NORRIS). *Dept. of Biochemistry, Univ. of Washington, Seattle.*

When crystalline yeast glyceraldehyde-3-phosphate dehydrogenase was used to catalyze the oxidation of glyceraldehyde in the presence of DPN and arsenate, it was noted that incomplete reduction of the coenzyme occurred and final stable optical extinction values at 340 mμ were not obtained. This was explained by the finding that $DPNH_2$ is unstable in the presence of the enzyme reacting to yield a product in which the 340 mμ absorption band is shifted to a lower wave length. The complete spectrum of the product appears identical to that of the product of non-enzymatic acid destruction of $DPNH_2$. The turnover number of the enzyme in this 'destruction' reaction is of a low order, and attempts to obtain a fraction from yeast juice more active than crystalline glyceraldehyde-3-phosphate dehydrogenase have not been successful. The reaction is inhibited by nicotinamide and by oxidized DPN. The latter finding would explain how it was possible for Meyerhof and co-workers to carry out equilibrium studies on the triose phosphate reaction catalyzed by the yeast enzyme in the presence of phosphate. The pH optimum for the $DPNH_2$ destruction reaction is also several pH lower than that usually employed in triose phosphate oxidation studies.

Reversed phase paper chromatography of steroids. THEODORE H. KRITCHEVSKY (introduced by KONRAD DOBRINER). *Sloan-Kettering Inst. for Cancer Research, New York City.*

The reversed phase method of paper partition chromatography on silicone treated paper has been applied to the separation of various groups of steroids including hydroxyl and ketonic derivatives of androstane, etiocholane, pregnane and allopregnane, cholesterol, bile acids, estrogens and metabolites of adrenocortical hormones. A method for the quantitative elution of the steroids has been developed for measurement by ultraviolet and infrared spectrometry. The procedures have been used for the separation, identification and quantitative determination of steroid metabolites found in urine and tissues.

Individual variations in serum protein bound iodine and in reduced glutathione in bovine blood. H. O. KUNKEL,* KENNETH H. BURNS* AND CARL M. LYMAN. *Texas A. and M. College System, College Station.*

Individuals of the bovine species vary in growth rate and productive ability. Studies have been initiated in order to study individual biochemical variations in the blood of young male beef cattle. Two constituents, serum protein bound iodine (thyroxine) and erythrocyte reduced glutathione (GSH) were found to be characteristic of the individual. Although the protein bound iodine generally varied with age and size of the animal, there was considerable variability (20–40% from mean) of values within groups of animals of a single beef breed, sex and weight group (±10% from mean). Serum protein bound iodine could be correlated (+.883) with resting metabolic rates taken 2–3 months after analysis of the sera. GSH of Hereford bulls 10–12 months of age varied from 18–33 mg/100 ml. of blood. Breeding and/or previous treatment imposed individual differences in blood GSH, which were relatively constant and remained a characteristic of the animal for several months even though the animals received a standard ration and were handled as similarly as possible. Possible significance of these individual variations will be discussed.

Photoelectric measurement of pH. GRANVIL C. KYKER, JOSÉ A. GOYCO,* MARÍA L. QUINTANA* AND ELBA IRIS PORRATA.* *Dept. of Biochemistry and Nutrition, School of Medicine, School of Tropical Medicine, Univ. of Puerto Rico, San Juan.*

Further study on the measurement of pH with photoelectric colorimeters, as introduced earlier by one of us, now includes calibration from pH 1.8 to 9.8 on 4 types of instruments and application to various materials. Calibration is empirical and is based on expressing the ratio of the densities of the colors of a two-color indicator as a function of pH. The density of each color is measured using the filter which approximates as nearly as possible the spectrophotometrically determined wave length of maximum absorption. Application consists of using a specimen as its own blank, adding the appropriate indicator, observing the color density with the two filters selected for the separate colors, taking the ratio of the densities, and using the ratio to read the pH from the calibration. The selected indicators are as follows: thymol blue (acid range), cresol red, bromphenol blue, bromcresol green, bromcresol purple, bromthymol blue, meta cresol purple, and thymol blue

(alkaline range). Numerous measurements on various fruit juices, urine, plasma, and titration curves indicate advantages for the method over ordinary colorimetric procedures but less precision than that which prevails with glass electrode instruments. When compared with the latter, a mean difference of 0.04 pH unit is characteristic of results on various juices. The advantages over visual colorimetry include greater accuracy, elimination of color standards, and independence of the concentration of indicator within wide quantitative limits.

Effect of ascorbic acid on oxidation of tyrosine. BERT N. LADU, JR. (introduced by BERNARD B. BRODIE). *New York Univ. Research Service, Goldwater Memorial Hosp., New York City, and Natl. Heart Inst., Natl. Inst. of Health, Bethesda, Md.*

An extract of rabbit liver acetone powder contains a series of enzymes which catalyze the oxidation of L-tyrosine to acetoacetic acid. Although this activity is almost completely lost upon dialysis of the extract, it can be largely recovered by the addition of α-ketoglutarate and ascorbic acid. Furthermore, the addition of ascorbic acid to the undialyzed extract greatly accelerates the rate of tyrosine oxidation, providing α-ketoglutarate is present to accelerate the transamination step. Our results indicate that the stimulatory effect of ascorbic acid is on the first oxidative step, the transformation of *p*-hydroxyphenylpyruvic acid to 2,5-dihydroxyphenylpyruvic acid. The oxidation of *p*-hydroxyphenylpyruvic acid is inhibited by high concentrations of oxalacetate or pyruvate, but this inhibition can be reversed by increasing the concentration of ascorbic acid. Dehydro-ascorbic acid, D-isoascorbic acid and hydroquinone also stimulate the rate of tyrosine oxidation, but dihydroxymaleic acid, catechol and resorcinol are ineffective. The oxidation of tyrosine is inhibited by low concentrations of diethyldithiocarbamate; however, this inhibition can be reversed by Cu^{++}. This suggests that copper may also be involved in this pathway of tyrosine oxidation.

Effects of thioacetamide on intracellular distribution of protein-nitrogen and pentosenucleic acid in rat liver. ANNA KANE LAIRD (introduced by J. A. MILLER). *McArdle Mem. Lab., Univ. of Wisconsin, Madison.*

Rather (*Bull. Johns Hopkins Hosp.* 88, 38, 1951) has demonstrated that a dietary supplement of thioacetamide results after a few days in a regular increase in hepatic nuclear volumes in powers of two, accompanied by an increase in nucleolar and cytoplasmic volumes. These changes are largely reversible. In the present study, livers of rats which had received daily subcutaneous injections of thioacetamide for variable periods of time were perfused with physiological saline, homogenized, and separated by differential centrifugation into nuclear, large granule, small granule, and supernatant fluid fractions. Protein-nitrogen and nucleic acids were determined in each fraction, and the numbers of nuclei per gram and per whole liver were determined. The analytical results were expressed as the amounts in the average cell and in the average whole liver. Despite the large increase in nuclear volumes, there was no change in the desoxypentosenucleic acid per nucleus, and therefore the process cannot be due to polyploidy. Changes in liver size induced by thioacetamide occur without change in the total desoxypentosenucleic acid or total cell number in the livers, and hence are due to changes in cell size. The nuclear protein-nitrogen and pentosenucleic acid of the average cell were significantly increased. The increases in nuclear and nucleolar volumes evident on microscopic examination, are therefore associated with increases in nuclear protein and pentosenucleic acid, but not desoxypentosenucleic acid. Marked increase in the total pentosenucleic acid of the whole liver occurred after prolonged administration of the drug.

Mammalian glutamotransferase. ABEL LAJTHA AND PAULA SCHWERIN (introduced by H. WAELSCH). *N. Y. State Psychiatric Inst. and Dept. of Biochemistry, Columbia Univ., New York City.*

The enzyme system which catalyzes the exchange of the amide group of glutamine with hydroxylamine occurs in all mammalian tissues studied (WAELSCH, *Federation Proc.*, 1951). Aqueous extracts of acetone-dried tissue powder require for activation phosphate and Mn^{++}. After partial inactivation by dialysis or other procedures, the enzyme may be reactivated by the addition of minimal amounts of AMP, ADP, ATP and various CoA preparations. In this respect the enzyme from mammalian sources is similar to the glutamotransferase from plant material (Stumpf) or Neurospora, but differs in that it cannot be completely inactivated by dialysis. Upon purification by ammonium sulfate and alcohol fractionation, and adsorption, the enzyme is no longer activated by AMP. The effect of amino acids and peptides on purified preparations of glutamotransferase from brain cortex and its properties are discussed.

Mechanism of nucleoside cleavage in *Lactobacillus pentosus*. J. O. LAMPEN AND T. P. WANG.* *Dept. of Microbiology, School of Medicine, Western Reserve Univ., Cleveland, Ohio.*

Previous studies (WANG, *Federation Proc.* 9: 399, 1950) with a nucleosidase preparation from *L. pentosus* showed that uridine and cytidine were

cleaved more rapidly in phosphate or arsenate buffers than in tris(hydroxy-methyl)aminomethane-HCl(TRIS) buffer. Subsequent experiments have demonstrated that the cleavage of purine nucleosides and of uric acid riboside was actually reduced by the addition of phosphate or arsenate. Only free ribose and the nitrogenous base could be identified as products from either purine or pyrimidine nucleosides. The extract did not catalyze the hydrolysis or arsenolysis of ribose-1-phosphate, nor could transglycosidation be demonstrated with uridine and cytosine or hypoxanthine. On reexamination of the pyrimidine nucleosidase, it was noted that the 'stimulatory' effects of phosphate and arsenate could be duplicated by sulfate or succinate. Pyrimidine nucleosidase has been found to be stable in the presence of divalent anions but to be inactivated rapidly in the presence of monovalent anions or in the absence of added anions. In contrast, purine nucleosidase is stable in TRIS buffer but rapidly becomes inactive in phosphate or arsenate buffers. Reactivation of either enzyme has not been achieved thus far. It is concluded that the nucleosidases of *L. pentosus* catalyze the hydrolysis of the nucleosides. The effects of phosphate or arsenate are probably a consequence of the differential stability of the enzymes in the various buffers.

Biosynthesis of squalene and cholesterol. ROBERT C. LANGDON (introduced by K. BLOCH). *Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.*

Normal rat tissue does not contain significant amounts of squalene. However, when this hydrocarbon is fed, a substantial fraction can subsequently be recovered from the liver and intestine. When natural squalene together with 1-C^{14} acetate or 2-C^{14} acetate was fed to rats, the hydrocarbon subsequently isolated from liver and intestine contained C^{14} in significant concentrations. This material, on the basis of isotope dilution and formation of the hexahydrochloride derivatives appears to be identical with squalene. This finding demonstrates the synthesis of this terpenoid hydrocarbon by animal tissues. Small quantities of C^{14} squalene isolated from one of the above experiments were fed to mice for 2 days and the tissue lipids were isolated. The specific activity of the pooled cholesterol from liver and intestine was 6-7% that of the squalene fed and 10% of the administered squalene C was recovered as tissue cholesterol from the whole animal. Purification of the sterol by way of the digitonide and dibromide did not reduce its C^{14} content. The saturated fatty acids from liver and intestine had a specific activity less than 10% that of the cholesterol from the same source. These results indicate an efficient conversion of squalene to cholesterol by a mechanism which does not involve C_2 intermediates.

Action of branching enzyme from liver on outer chains of polysaccharides. JOSEPH LARNER (introduced by GERTY T. CORI). *Dept. of Biochemistry, Washington Univ. Med. School, St. Louis, Mo.*

After short incubation of amylopectin with a dialyzed liver protein fraction which had been freed of α-amylase, the iodine color was found to have changed from purple to reddish-brown. It was tentatively assumed that a branching enzyme had acted on the long outer branches of amylopectin. In order to obtain definite proof, an artificial polysaccharide was prepared from glycogen by lengthening the outer branches with additional glucose residues in 1–4 linkage by means of phosphorylase and C^{14} glucose-1-phosphate. After isolation of this polysaccharide, the liver brancher was allowed to act on it, and the polysaccharide was re-isolated. It was then degraded by the combined action of phosphorylase and amylo-1,6-glucosidase and the glucose liberated by the latter enzyme isolated as described previously (*J. Biol. Chem.* 188: 17, 1951). The glucose which is derived solely from the residues at branch points was expected to have considerable radioactivity, provided branching had taken place. This was actually found. In control experiments, ptyalin was substituted for the liver fraction, and the polysaccharide degraded 2–3%. The glucose isolated after the degradation with phosphorylase plus glucosidase was found to be devoid of radioactivity, showing that ptyalin under the conditions of the experiment had no branching action. Since the liver brancher acted in the absence of added phosphate, it may be regarded as a transglucosidase which exchanges 1–4 for 1–6 glucosidic links. It probably transfers short chains of glucose residues. The specificity requirements of the enzyme are now being studied.

Separation of the porphyrins by chromatography. EARL G. LARSEN* AND JAMES M. ORTEN. *Dept. of Physiological Chemistry, Wayne Univ. College of Medicine, Detroit, Mich.*

The use of readily available 'analytical reagent' silicic acid in the separation of the methyl esters of the naturally occurring porphyrins by partition chromatography with hydrated silica gel has been investigated. Columns varying from 5–16 mm. in diameter and containing from 1–25 gm. of silicic acid were found to give excellent resolution of the porphyrins which could then be eluted with mixtures of chloroform in ligroin and crystallized. The procedure has the advantages over earlier methods employed in this laboratory (LUCAS AND ORTEN, *J. Biol. Chem.*, 191; 287, 1951) of not re-

quiring laborious methods for preparing silicic acid, the ready availability of amounts of silicic acid sufficient for relatively large scale preparations, the smaller particle size of the adsorbent and thus larger surface area, and an adequate resolution without acid impregnation of the silica gel. No appreciable diffusion at the boundaries of zones in static columns was observed thus indicating the importance of physical adsorption in the resolution of the porphyrins by partition chromatography on hydrated silica gel under these conditions. Further comparative studies using ion exchange resin columns for the separation of the free porphyrins are in progress and will be discussed.

Fractionation of proteins from chicken erythrocyte nuclei. M. LASKOWSKI AND VIRGINIA KUBACKI ENGBRING.* *Dept. of Biochemistry, Marquette Univ. School of Medicine, Milwaukee, Wis.*

Chicken erythrocyte nuclei were digested with an excess of crystalline desoxyribonuclease at pH 7, and were centrifuged. The supernatant contained mainly the products of digestion of DNA, some other P containing substances and only a small amount of probably very numerous proteins, precipitable with trichloroacetic acid. The residue was extracted with 0.1 N HCl and recentrifuged. The supernatant was composed principally of histones which were precipitated at pH 7 and 70% saturation of $(NH_4)_2SO_4$. The electrophoretic analysis revealed the presence of numerous, apparently closely related, histones. Neither proteins of the albumin or globulin types, nor the protamines were present (Mirsky's reaction). The residue from the acid extraction was then extracted with alkali, adjusted to pH 8.5 and filtered. The filtrate was adjusted to pH 5 which resulted in the formation of a precipitate which was soluble in alkali and slightly soluble in acid. It gave positive color reactions for proteins, including the reaction for tryptophan; the Molisch test was also positive. The ultraviolet spectrum was atypical, suggesting a conjugated protein. The substance showed a uniform electrophoretic pattern and a uniform boundary in the ultracentrifuge. In collaboration with Dr. J. M. Luck and his group similar extractions were carried out on nuclei from liver and thymus. An alkali soluble proteinic substance was obtained in both cases. Neither of these two was electrophoretically identical with the corresponding substance from erythrocyte nuclei, but all three were very similar in respect to their solubility and gross composition.

Formaldehyde metabolism in propionic acid fermentation. FREDRICK W. LEAVER (introduced by HARLAND G. WOOD). *Dept. of Biochemistry Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

It has previously been shown that during the fermentation of various substrates by *Propionibacterium arabinosum* the carbon of formaldehyde is incorporated into every position of propionate (*J. Am. Chem. Soc.* 72: 5326, 1950) and with glycerol-1-C^{14} that formaldehyde is formed mainly from the number one carbon (*J. Am. Chem. Soc.* 73: 2974, 1951). Further study with $HC^{14}HO$ has shown that the ethylene carbons of succinate have a specific activity approximately twice that of the ethylene carbons of propionate. We have further studied the source of formaldehyde from C^{14}-glucose using proliferating cells and trapping the formaldehyde with dimedon (WOOD AND WERKMAN, *J. Bact.* 30: 652, 1935). The isolated formyl-dimedon was recrystallized to constant activity and identified by its mixed melting point. From totally labeled glucose the formyl-dimedon had a specific activity of 95% that of the original glucose which indicated that glucose is the primary source of formaldehyde; from glucose-1-C^{14} the activity was 2.7% of that of the one carbon, and from glucose-3,4-C^{14} it was 23% of the 3,4 carbons. It thus appears likely that the 2,5 and/or 6 carbons are the major sources of formaldehyde. Since glycerol-1-C^{14} gave highly labeled formaldehyde it seems possible that the sixth carbon of glucose is the major source of formaldehyde. If this should prove to be the case, it would be necessary to assume that in part at least the metabolism of the 1 and 6 carbons of glucose is different.

Effect of 2,4-dinitrophenol on turnover of adenosinetriphosphate. KWAN-HUA LEE* AND JOHN J. EILER. *College of Pharmacy, Univ. of California, San Francisco.*

The turnover of ATP in fluoride containing rat brain homogenates (ELIER AND MCEWEN. *Arch. Biochem.* 20: 163, 1949) to which pentobarbital had been added to limit the oxidation of succinate to the stage of fumarate was studied with radioactive inorganic phosphate at 17°. With no phosphate acceptor, and in the presence or absence of 1×10^{-5}M 2,4-dinitrophenol (DNP), the concentrations of ATP and inorganic phosphate remained constant. The rate of exchange of phosphate (V) followed the equation: $V = \ln \frac{Ao}{A-B} \cdot \frac{ab}{a+b} \cdot \frac{1}{t}$; wherein A and a represent the specific activity and the amount of inorganic phosphate, respectively; and B and b represent the specific activity and the amount of the labile phosphate of ATP, respectively. The relation between V, A and B, during the course of the equilibration of radioactivity was such as to

demonstrate that the two labile phosphates of ATP were equally active. The rate of oxygen uptake, with and without DNP, was constant at 1.4 μ atoms of oxygen/minute (6 ml. reaction mixture). This value with the values for V, yielded a calculated P/O of 1.0 for the oxidation of succinate to fumarate, in the absence of DNP, and a P/O of 0.6 in the presence of DNP. Under anaerobic conditions there was little exchange of radioactivity and only a slow rate of decrease of labile phosphate (less than 10% of the value of V under oxygen). (Supported in part by a USPHS (NIH) Grant.)

Fractionation of pig complement. MYRON A. LEON,* OTTO J. PLESCIA,* MICHAEL HEIDELBERGER AND ROBERT S. GORDON, JR.* *Dept. of Medicine, College of Physicians and Surgeons, Columbia Univ., and Presbyterian Hosp. New York City.*

Purified components of complement are essential for elucidating the mechanism of the action of complement in immune processes. Fractionation of pig complement was therefore undertaken. The details of procedures of fractionation based on differences in solubility and electrophoretic behavior will be discussed.

Blood pressure of bilaterally nephrectomized dogs. JACK R. LEONARDS AND CHARLES R. HEISLER.* *Dept. of Biochemistry, Western Reserve Univ., Cleveland, Ohio.*

Convincing data showing that hypertension can develop in nephrectomized dogs has been presented by Grollman and co-workers. However, no significant increase in blood pressure was obtained in such dogs in our laboratory when the animals' lives were prolonged by feeding electrolyte-free diets, glucose intravenously or treatment with the artificial kidney. In order to resolve this apparent discrepancy additional studies were carried out on dogs kept alive by peritoneal lavage. In one group of 10 animals fluid balance was reasonably well maintained and their weight either remained the same or decreased a little. These animals survived from 9–22 days and in no case was there any significant increase in the mean blood pressure as measured with a mercury manometer by direct femoral artery puncture. Ten similar animals were gradually overloaded with fluid. Their weight increased by an average of 1.3 kg. The blood pressure of all of these dogs increased by at least 40 mm. Hg and in 8 of the 10 animals the increase amounted to 60–80 mm. These overhydrated animals did not have any observable edema. Blood volumes (Evans Blue) were measured in 4 of these animals and were not increased. However, the inulin space ('extracellular fluid volume') was doubled. The intramuscular injection of 40 mg. of ACTH/day in 3 divided doses did not result in an increase in blood pressure in nephrectomized dogs that were not overhydrated.

Separation of a cysteine-activated proteinase from culture filtrates of Clostridium histolyticum. IRWIN H. LEPOW, SIDNEY KATZ AND JACK PENSKY (introduced by LOUIS PILLEMER). *Inst. of Pathology, Western Reserve Univ., Cleveland, Ohio.*

A proteolytic enzyme from culture filtrates of *Cl. histolyticum* has been highly purified by fractionation in methanol-water mixtures of controlled pH, ionic strength, protein concentration and temperature. The proteinase in the parent filtrate is activated by cysteine and further activated by cysteine plus ferrous ions (WEIL AND KOCHOLATY, *Biochem. J.* 31: 1255, 1937). Purified preparations, however, are optimally activated by cysteine alone. The final preparation is essentially free of lethal and necrotizing toxin and of collagenase activity. Details of assay, purification and activation behavior will be presented.

Quantitative estimation of acetone bodies by means of alkaline vanillin reagent. VICTOR E. LEVINE AND MICHAEL TATERKA.* *Creighton Univ. School of Medicine, Omaha, Nebraska.*

Vanillin in high concentrations of alkali reacts with many carbonyl compounds in aqueous or alcohol solution producing an intense yellow complex. Acetone can be separated from non-volatile ketones, such as pyruvic acid, and estimated quantitatively by the vanillin reaction. Urine (10 ml.) or protein-free blood filtrate (10 ml.) is acidified with 5 drops of 10% sulfuric acid and distilled in the Stotz apparatus (*J. Biol. Chem.* 148: 585, 1943) into 1 ml. of 0.5% sodium bisulfite. One ml. of the distillate is treated with 1 ml. 0.05 M aqueous vanillin and 2 ml. 10 N sodium hydroxide. The contents are placed in a water bath at 60°C. for 15 minutes, cooled and made up to 6 ml. with distilled water. Transmittance is measured in an Evelyn electrophotometer using a 420-millimicron filter. A standard calibration curve is made with acetone sodium bisulfite. The method, sensitive to 2 gamma acetone/ml., follows Beer's law in ranges from 2–100 gamma/ml. The vanillin reaction is more reliable for ketones, which do not polymerize, than for aldehydes. It is unreliable for carbohydrates due to the effect of alkali. Ketones with one radical as a methyl group are more reactive than radicals with more than one carbon atom. Reactivity diminishes with an increasing number of carbons in the radicals. The ketone, 10-non-adecanone, $CH_3(CH_2)_8$-$CO(CH_2)_8CH_3$, proved non-reactive. A phenyl group weakens the reaction. When both radicals are phenyl groups, as in benzophenone, no reaction takes place.

Histidine synthesis in yeast and human liver. LOUIS LEVY AND MINOR J. COON (introduced by D. WRIGHT WILSON). *Dept. of Physiological Chemistry, Univ. of Pennsylvania School of Medicine, Philadelphia.*

Evidence has been presented that formic acid gives rise to carbon 2 of the ring of histidine in *S. cerevisiae* and that the imidazole ring of the amino acid results from a series of reactions different from those which lead to the imidazole ring of purines (*J. Biol. Chem.* 192: 807, 1951). Further studies have been undertaken to test the possibility that glutamic acid is the source of the carbon chain of histidine. A culture of yeast was allowed to grow on a medium containing non-radioactive L-glutamic acid and uniformly-labeled glucose. The protein fraction of the harvested yeast was hydrolyzed, and the resulting amino acids were fractionated with the aid of ion exchange resins. The purified histidine was highly radioactive, demonstrating that the amino acid was formed quite directly from glucose. This finding and the results of other studies employing glucose-1-C^{14} suggest that neither glutamate nor other 5-carbon compounds derived from it are involved in the biosynthesis of histidine. Further evidence supporting this view was obtained from an experiment in which it was demonstrated that the methyl carbon of acetate can furnish carbon 2 of the ring of histidine, presumably via formate, but that acetate and compounds derived from it such as glutamate, do not contribute to the formation of the 5-carbon chain of histidine. In other studies, human liver slices were incubated with formate-C^{14} and found to produce a labeled compound which was identified by paper chromatography as histidine.

Denaturation of bovine serum albumin. MILTON LEVY AND ROBERT C. WARNER. *Dept. of Chemistry, New York Univ. College of Medicine, New York City.*

The rate of denaturation (followed through solubility) of crystalline bovine serum albumin has been studied over a wide range of temperature and pH. The reaction kinetics observed are dependent on the conditions used for precipitation of the products. Suitable conditions were found for first order kinetics from pH 0.8 to about 5. The rates are minimal at about pH 2 and maximal near 4.5–5. The temperature coefficient of rate varies from zero in the region pH 3–4 to high values at pH <2. Alkaline to pH 5 the rate falls off from that required by the first order equation as the reaction proceeds. A minimum rate is observed at pH 7–8. In the acid region the data are accommodated by the hypothesis of a limited number of prototropic hydrogen bonds acting as structural centers (LEVY AND BENAGLIA, *J. Biol. Chem.* 186: 829, 1950). In the alkaline region the kinetics are not sufficiently clear for further interpretation. With low salt concentrations in the precipitating buffers the reaction shows a lag period. The reaction rate can be described tentatively as the result of the first order formation of a soluble product which dimerizes or polymerizes to an insoluble product.

Effect of modifications of bedside test on its correlation with the one-stage prothrombin test. DANIEL W. LEWIS* AND MURIEL PLATT MUNRO. *Dept. of Medicine and Charlotte Drake Cardeza Fndn., Jefferson Med. College and Hospital, Philadelphia, Pa.*

Previous work (*J. Lab. Clin. Med.* 35: 16, 1950) has shown a correlation between bedside and one-stage prothrombin times using a commercial thromboplastin. Modifications have been introduced in the bedside test in an attempt to improve this correlation. The bedside test was diluted so that the plasma concentration was equivalent to that in the actual clotting mixture of the one-stage test (27%). Shorter clotting times were frequently obtained but relatively little improvement in the correlation resulted. Dilution of the bedside test mixture introduced the problem of calcium concentration. Two concentrations of calcium have been tried. The addition of calcium to the diluting fluid equivalent to the ionic calcium concentration of plasma (4.3 mg. %) was found to give shorter clotting times than 10 mg. %. Mann *et al.* (*Am. J. Clin. Path.* 20: 225, 1950) report that the incubation of thromboplastin with dilute human serum markedly shortens the one-stage prothrombin times of plasma from patients receiving Dicumarol. Human serum has been found to affect the bedside clotting time much less than the one-stage clotting time. Variations in the thromboplastin appear to have less effect on the bedside test than they do on the one-stage test. It would appear that a carefully controlled bedside test probably offers fewer hazards in the control of Dicumarol therapy than does the one-stage test.

Maintenance on a diet low in fat. GEORGE T. LEWIS, DONALD W. SMITH,* PATRICK V. FERRO* AND JAMES J. GRIFFITTS.* *Med. Research Fndn. of Date County, Miami, Fla.*

A feeding solution has been devised which is designed to be administered by continuous drip through a nasal tube. The solution provides an adequate caloric intake, nearly completely hydrolyzed protein, and a considerable amount of carbohydrate, but little if any fat. Patients have been maintained on this material with only a vitamin supplement for long periods, in one case for 14 months. Analyses will be reported of blood lipid

fractions found during this period on a nearly fat-free diet. Duodenal secretion has been examined and its enzyme content found to correspond to the need for digestion of the ingested feeding solution. Results of a fat tolerance test carried out under these circumstances will be presented. The question of the necessity of fat in the diet will be discussed.

Preparation of a biologically purified and clinically active ACTH from horse pituitary glands. CHOH HAO LI. *Dept. of Biochemistry, Univ. of California, Berkeley.*

Horse pituitary glands have been found to be a good source of adrenocorticotropic hormone (ACTH). By the method recently developed for the preparation of sheep and pig ACTH (LI, LIDDLE, REINHARDT AND BENNETT, *Proc. Soc. Exper. Biol. & Med.*, Dec. 1951), the ACTH content in whole horse pituitary glands may be almost quantitatively recovered. From 1 kg. of fresh whole horse pituitaries, 20 gm. of acid-acetone powder (AAP) was obtained. Two gm. of AAP was dissolved in 100 cc. pH 3.0 solution, and 6 cc. saturated NaCl solution was added. After the removal of the precipitate, solid NaCl was added to the supernatant to saturation. The saturated NaCl precipitate was next suspended in water and dialysed until salt-free. The dialysed solution was made up to 0.2 N HCl, and the resulting acidified solution was kept in a boiling water bath for 60 minutes. After cooling, 6 M NaOH solution was added until the pH reached about 3.0. The pH 3.0 solution may either be lyophilized or used directly. The final product contains 600–700 mg. ACTH having a potency equivalent to the international standard as determined by the ascorbic acid depletion method of Sayers *et al.* (*Endocrinology* 42: 379, 1948). The material was found to be free from other anterior and posterior pituitary hormones when it was tested by the conventional techniques for the determination of these hormones. Clinical investigations in a patient with rheumatoid arthritis indicated that the horse ACTH prepared by the method herein described is therapeutically and metabolically effective.

Metabolic role of vitamin B_{12}. C. T. LING* AND BACON F. CHOW. *Dept. of Biochemistry, School of Hygiene and Public Health, Johns Hopkins Univ., Baltimore, Md.*

In our previous studies on the metabolic role of vitamin B_{12} it was shown that the carcass compositions of rats raised on a soybean meal diet were low in fat, high in water, but normal in protein contents. The sulfhydryl content of their blood cells was likewise low. Injection of vitamin B_{12} rapidly corrected these abnormalities. These investigations were now extended to ascertain *1*) whether similar changes in sulfhydryl content could occur in the blood cells of patients with pernicious anemia and in those of rats with anemia due to iron deficiency; *2*) whether B_{12}-deficiency entails derangement in carbohydrate metabolism; and *3*) any possible interrelationship between these two biochemical changes. Our experimental results demonstrate that administration of vitamin B_{12} to patients with pernicious anemia as well as to vitamin B_{12}-deficient rats resulted in a marked increase in SH-content of the blood cells, primarily due to glutathione, and in blood phospholipids. Decrease in glutathione of blood cells of rats could also be brought about with a high carbohydrate diet coupled with injections of glucose but now with an iron deficient diet. The effect of high-carbohydrate diet was conceivably due to increased utilization or requirement of B_{12}. The hypothesis that the requirement of vitamin B_{12} is related to carbohydrate intake is supported by our findings that B_{12}-deficient rats had smaller carbohydrate reserve, and intravenously administered glucose was poorly utilized by these animals.

Enzymatic formation of monosaccharides from hyaluronic acid. ALFRED LINKER* AND KARL MEYER. *Dept. of Medicine, Columbia Univ. College of Physicians and Surgeons, and the Edward Daniels Faulkner Arthritis Clinic of the Presbyterian Hosp., New York City.*

Hyaluronic acid yields on hydrolysis, by purified testicular hyaluronidase, a mixture of oligosaccharides. These are partially hydrolysed to N-acetylglucosamine and glucuronic acid by crude testicular and liver extracts, containing β-glucuronidase and β-glucosaminidase (*J. Biol. Chem.* 192: 275, 1951). Liver extract freed of β-glucosaminidase yields glucuronic acid as the only monosaccharide. A similar result is obtained on short incubation with crude extracts. These findings indicate that β-glucuronidase hydrolyses only terminal glucuronidic bonds. N-acetylglucosamine is evidently a product of the action of β-glucosaminidase. By paper chromatography and analysis, one of the intermediate hydrolysis products has been shown to be a trisaccharide, composed of two molecules of N-acetylglucosamine and one molecule of glucuronic acid. The glucosaminidase apparently hydrolyses this trisaccharide, giving N-acetyl-glucosamine and a disaccharide, glucuronido-N-acetylglucosamine, which is resistant to hydrolysis. In related experiments, the oligosaccharides formed on digestion of chondroitin sulfate A with purified testicular hyaluronidase were likewise shown to be hydrolysed by crude testicular extract. The only monosaccharide found in this reaction was glucuronic acid. (This work

was supported by grants from the U. S. Public Health Service and from the Helen Hay Whitney Foundation.)

Influence of protein concentration upon electrophoretic mobility of serum proteins. RICHARD W. LIPPMAN AND JAY BANOVITZ (introduced by ERWIN HAAS). *Inst. for Med. Research, Cedars of Lebanon Hosp., Los Angeles, and the Gates and Crellin Labs. of Chemistry, California Inst. of Technology, Pasadena.*

Previous investigators have reported the change in distribution of serum protein fractions, observed in electrophoresis patterns, with a change in ionic strength (PERLMANN AND KAUFMAN, *J. Am. Chem. Soc.* 67: 638, 1945) or with a change in protein concentration (*idem, ibid.*; JAMESON, *Arch. Biochem.*, 15: 389, 1947). In this study we investigated the effect of protein concentration upon mobility of serum proteins, other conditions remaining constant. Experiments were performed with rat serum in veronal buffer, ionic strength 0.1, pH 8.6, in an open electrode system. Serial dilutions of serum were dialyzed against the buffer. The mobility of all the serum proteins in the descending limb increased about 33% as the protein concentration fell from 5.5%–0.5%. The relationship of mobility to protein concentration was approximately linear below 3%. The effect was of much smaller magnitude in the ascending limb than in the descending limb. The previously mentioned change in apparent relative proportion of the serum protein fractions with dilution was confirmed. Experiments with purified bovine serum albumin, homogeneous by electrophoresis, were performed under the same conditions, but with an electrode system closed on one side. Similar, though smaller, changes in mobility were observed. In order to compare mobilities the protein concentration, in addition to other factors, must be held constant, or the values must be corrected to a constant concentration. The effects observed may be the result of interactions between protein molecules in concentrated solution which would reduce the effective charge.

Oxidative phosphorylation with soluble extracts. J. W. LITTLEFIELD AND D. R. SANADI (introduced by M. JOHNSON). *Inst. for Enzyme Research, Univ. of Wisconsin, Madison.*

The dismutation of alpha-ketoglutarate to succinate and glutamate can be coupled to esterification of inorganic phosphate in a completely soluble system. Among other things this dismutation appears dependent on coenzyme A, inorganic phosphate, ADP, and an enzyme extracted from pig heart and purified by alcohol and ammonium sulfate fractionation. At least one molecule of inorganic phosphate is esterified for each molecule of alpha-ketoglutarate oxidized. In the earlier studies on oxidative phosphorylation by Green and Beinert in this laboratory the alpha-ketoglutaric dehydrogenase used was particulate while the phosphorylation enzyme was in the soluble fraction. Phosphorylation by this purified enzyme in other oxidative systems will also be reported on.

Chemical changes in normal and neoplastic growth. M. E. LOMBARDO,* D. V. N. REDDY,* P. MCCARTHY,* E. SINGER* AND L. R. CERECEDO. *Dept. of Biochemistry, Fordham Univ., New York City.*

It has been shown in this laboratory that there is a significant increase in the nucleic acid concentration in the tissues of the host during tumor development. The increase in pentose nucleic acid (PNA) and desoxypentose nucleic acid (DNA) is not restricted to such active organ as the liver but is also found in the lung, kidney, thymus and lymph nodes. The chemical changes during regression of a transplanted lymphosarcoma were also studied. The regression was produced by the administration of desoxypyridoxine to animals receiving a vitamin B_6-deficient diet. Significant increases in nucleic acids were found in the tissues of mice receiving desoxypyridoxine on a diet rich in vitamin B_6. Similar changes were also found in mice receiving desoxypyridoxine on a vitamin B_6-deficient diet. In the latter animals, however, there was a pronounced drop in DNA in the spleen. A comparative study of the mouse and the chick embryo revealed some fundamental differences in the mammalian and the avian embryo with regard to their nucleic acid content. At all stages of growth investigated the concentration of DNA is higher than PNA in the mouse embryo, whereas in the chick embryo the reverse is true. Values for the amino acid changes in normal and neoplastic growth will also be presented. (This investigation was supported in part by grants from the National Cancer Institute, the National Institute of Health, Public Health Service, and the Damon Runyon Memorial Fund.)

Heme synthesis in non-intact mammalian and avian erythrocytes. IRVING M. LONDON AND MARTHA YAMASAKI (introduced by D. RITTENBERG). *Dept. of Medicine, Columbia University College of Physicians and Surgeons, New York City.*

Previous studies have demonstrated that immature non-nucleated mammalian erythrocytes and avian erythrocytes are capable of synthesizing heme *in vitro* (*J. Biol. Chem.* 183: 749, 757, 1950). To investigate the enzymatic mechanisms involved in heme synthesis, it is desirable that the factor of permeability of the erythrocyte membrane to metabolic agents be eliminated. On incubation with 1-C^{14}-sodium acetate or with 2-C^{14}-glycine the hemolysates of immature rabbit

erythrocytes or of duck erythrocytes which have been lysed by distilled water form C^{14} labeled heme. The optimal conditions for heme synthesis in these non-intact erythrocyte systems are under investigation. The addition of sucrose to restore isotonicity of the duck hemolysate enhances the rate of heme synthesis. A similar degree of enhancement has not been observed with isotonic concentrations of glucose, fructose or raffinose. With varying conditions, the rates of heme synthesis in the hemolysates have ranged from about 15% to 70% of the rates observed in aliquots of intact erythrocytes. The effects of cation concentrations, and of agents such as folic acid and its antagonists, vitamin B_{12}, and lead on heme synthesis in the hemolyzed preparations will be described. 2-4-Dinitrophenol in a concentration of 1×10^{-4}M produced about 50% inhibition of heme synthesis in the hemolysate of duck erythrocytes. Fractionation studies are in progress to determine the cellular constituents concerned with heme synthesis.

Nature of ribonuclease action. HUBERT S. LORING AND ARTHUR P. RINFRET.* *Stanford Univ., Calif.*

The hydrolysis of sodium ribonucleate by ribonuclease was studied over a 72-hour period with and without Mg^{++} in comparison with suitable controls. The rate of liberation of acid groups titrating at pH 8 was determined in each case; and at intervals during the hydrolysis aliquots were removed, fractionated with Al^{+++} at pH 3 into an Al^{+++} soluble (Al-sol) fraction which was analyzed for total phosphorus (P), nitrogen (N), purine N, and pyrimidine components. The results show that after 4–8 hours about the same amount of nucleotides were made soluble by enzyme action both with and without Mg^{++}. Subsequently the amount of soluble nucleotides remained constant in the absence of Mg^{++} but decreased significantly where Mg^{++} was present. Comparison of acid liberation with the amount of soluble nucleotides formed showed that approximately one half of the total acid liberated by the enzyme was freed in 4–8 hours in the presence of Mg^{++}, forming products which are average dinucleotides. Subsequently there was a progressive liberation of acid groups approaching 1 equivalent/mole of Al-sol P in the presence of Mg^{++} and 0.5 in its absence and corresponding to mononucleotides and average dinucleotides respectively. With Mg^{++} the decrease in the purine content of the Al-sol fraction after 72 hours amounted to 21% and in the pyrimidine content to 10% of the respective maximum values. The latter results can be best explained by a recombination of soluble nucleotides with the enzyme-resistant fraction and their subsequent precipitation as Al-insol components. Fractionation of the Al-sol fraction without further hydrolysis and the Al-insol fraction after alkaline hydrolysis resulted in the isolation of only the cytidylic acid isomer $[\alpha]_D + 50°$ in each case. The possible significance of the above results to nucleic acid structure will be discussed.

Amino acid incorporation by liver proteins. J. MURRAY LUCK AND ROBERT BRUNISH.* *Dept. of Chemistry, Stanford Univ., Stanford, Calif.*

Experiments, *in vitro*, demonstrated that desoxypentosenucleohistone (DNH) and histone prepared therefrom 'incorporate' amino acids more markedly than other liver proteins. Zero-time controls were negative: incorporation could seldom be detected with reaction times of less than 5 minutes. Incorporation by DNH was observed over the pH range 5.8–9.0 or higher but not at pH 1.0 or 4.8. Incorporation by histone increased with temperature; at 100° it was 6-fold that at 37°. Preheating at 100° for 10 minutes lessened somewhat subsequent incorporation at 37°. 'Incorporated' amino acids could not be removed by repeated washing with TCA, solvation in 1 N NaOH and reprecipitation, dialysis against non-isotopic amino acid, or reaction with ninhydrin (in the case of carboxy-labelled amino acids). Studies, *in vivo*, were carried out with phenylalanine-3-C^{14} and alanine-1-C^{14}. The half life of total liver protein was found to be about 6½ days. Histone and total nuclear proteins had slightly lower turnover rates. That of histone, however, was considerably higher than that of liver DNA as calculated from comparable studies by others with phosphorus and adenine. Histone was prepared from liver DNH obtained from animals sacrificed 2 hours after administering phenylalanine. The histone was dialyzed, precipitated and washed. Ninety per cent of the radioactivity was present in the phenylalanine isolated from the hydrolysis products. (Supported by a grant from the U. S. Public Health Service.)

Chemistry of bone formation: relationship between composition of inorganic precipitates and their supernatant solutions. RODERICK P. MACDONALD* AND ADRIAN C. KUYPER. *Wayne Univ. College of Medicine, Detroit, Mich.*

Precipitates were formed from inorganic salt solutions of the same composition as blood ultrafiltrate, except for slightly increased levels of calcium and phosphate. They were allowed to equilibrate at physiological temperature, pH, ionic strength, and carbon dioxide tension, for varying periods of time. The composition of the precipitates with respect to their content of calcium, phosphorus, carbonate and magnesium was found to vary with the composition of the supernatant solutions. An increase in bicarbonate content or a

decrease in phosphate or calcium content of the supernatant solutions is accompanied by an increase in the carbonate content of the precipitate. The composition of the precipitate may be expressed in terms of an equation, as a function of the degree of saturation of the supernatant solution with respect to calcium, magnesium, carbonate, and phosphate.

Transfer of N and P from parent T_7 bacteriophage to its progeny. R. P. MacKal* and L. M. Kozloff. *Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.*

Bacteriophage T_7 was labeled by growth on *E. coli* in synthetic culture medium containing $N^{15}H_4Cl$ and $Na_2HP^{32}O_4$. The labeled virus was concentrated and purified by differential centrifugation until the virus preparation was free of non-viral isotope and was homogeneous in the ultracentrifuge. Bacteria in isotope-free synthetic medium were infected with labeled virus producing a single generation of virus progeny. In agreement with the results obtained with T_6r+ only a small fraction (20–30%) of the N^{15} and P^{32} of the infecting parent T_7 was found in the virus progeny in the lysate. The remaining N^{15} and P^{32} of the parent virus nucleoprotein was found either in the bacterial debris or in the non-sedimentable fraction of the lysate. A considerable fraction of the breakdown of the virus occurred during the first few minutes following adsorption. To determine the nature and specificity of the parent contribution, experiments were performed in which prior to infection, the labeled phage was irradiated with sufficient UV light to reduce the virus titer by a factor of 10^9. When bacteria were infected with a mixture of irradiated labeled phage and live unlabeled phage, the same amount of isotope was found in the progeny as in control experiments. A similar result had been found previously for the distantly related phage T_6r+. Apparently the inheritance of parental N and P by a virus offspring is not an essential feature of the reproductive mechanism, but probably results from the non-specific use of breakdown products of the parent particle for the synthesis of the new virus.

Formation of formaldehyde in the biological oxidation of dimethylaminoethanol, dimethylglycine, sarcosine and methanol. Cosmo G. MacKenzie, John M. Johnston* and Wilhelm R. Frisell.* *Dept. of Biochemistry, Univ. of Colorado Med. School, Denver.*

In the course of further investigations on the production and metabolism of one carbon compounds, it has been found that dimethylaminoethanol and dimethylglycine are sources of formaldehyde. The substrates were incubated with homogenized liver or the particulate or supernatant fractions obtained by centrifugation at 2200xg in 0.075 M potassium phosphate containing Mg and Ca. Formaldehyde was isolated from the reaction mixture and characterized as the dimedon derivative. With dimethylaminoethanol, formaldehyde was obtained from the whole liver homogenate incubated at pH 8.8, but not at pH 7.8. Formaldehyde was not isolated when this substrate was added to either the supernatant or the particulate fractions alone. The reconstituted homogenate yielded formaldehyde, indicating that at least two components are involved in the conversion of dimethylaminoethanol to formaldehyde. In the case of dimethylglycine, incubation with the whole homogenate at either pH 7.8 or pH 8.8 produced formaldehyde. On the basis of the procedures employed, the enzyme system was located in the particulate fraction prepared by repeated centrifugation. More formaldehyde was obtained with this fraction alone than with the whole homogenate and none was obtained with the supernatant. The system that oxidizes the methyl group of sarcosine to formaldehyde was found to have a similar distribution. Both sarcosine and dimethylglycine were oxidized to formaldehyde by liver mitochondria prepared in 0.25 M sucrose according to the procedure of Schneider and Hogeboom. In contrast to the above compounds, methanol was oxidized to formaldehyde by the supernatant fraction, or the whole homogenate, but not by the particulate fraction.

Turnover rate of plasma fibrinogen. Robert E. Madden* and R. Gordon Gould. *Rush Dept. of Biochemistry, Presbyterian Hosp., and Dept. of Biological Chemistry, Univ. of Illinois College of Medicine, Chicago.*

The turnover rate of plasma fibrinogen was measured both in dogs and humans. $Sulfur^{35}$, either in synthetic DL-methionine or yeast cells, was fed, and turnover measured by assaying fibrin clots for the radioisotope. Two techniques were employed: *1*) following increasing fibrin specific activity during a period of feeding a constant daily dose, and *2*) following decreasing specific activity after a single large dose or period of constant daily feeding. In the latter technique it was found important to feed supplemental inert S-containing amino acids to obtain a true exponential dieaway curve by preventing reutilization of isotope. The 50% turnover value for dogs was 4.2 days, based on 9 experiments, and for humans 5.6 days based on 3 experiments. The 50% turnover value for total serum proteins in the dog was 11.4 days, based on 4 experiments. The rate of incorporation of S^{35} into fibrinogen was also studied; after a single 500 microcurie dose of labeled yeast, given orally to patients, the peak

value for specific activity of fibrinogen was reached in about 24 hours. It corresponded to 0.397 microcuries/gram of fibrinogen or a total yield in plasma fibrinogen of 0.7% of the administered dose. When a dog was bled 35% of its blood volume acutely during a dieaway period, the specific activity of the plasma fibrinogen decreased only slightly faster during subsequent days than in control animals. This suggests an extravascular pool of labeled fibrinogen or other plasma fibrinogen precursor available for replacement.

Cytochrome c reductase (DPN) from heart. HENRY R. MAHLER, LEO P. VERNON AND N. K. SARKAR (introduced by L. J. TEPLY). *Inst. for Enzyme Research, Univ. of Wisconsin, Madison.*

Hayaishi (Abstracts of 118th meeting of Am. Chem. Soc., Chicago, Ill. Sept. 1950) and Edelhoch, Hayaishi and Teply (in preparation) have described the conditions for preparing a soluble enzyme from pigeon breast muscle which catalyzes the reduction of ferricytochrome *c* by reduced diphosphopyridine nucleotide (DPNH). Cytochrome reductase (DPN) has now been isolated and purified from pig heart in good yield by the following steps: extraction of microsomal particles with K_2HPO_4, solubilization with 10% ethanol at 42°, and acid and alkaline ammonium sulfate fractionation. The homogenous enzyme shows the properties of a typical flavoprotein with a molecular weight of 75,000–80,000 calculated both from physicochemical constants and from flavin content. The enzymatic reaction involved has been studied in some detail and the stoichiometry, Michaelis constants and activation energy determined. The effects of several inhibitors, including antimycin and BAL, the probable nature of the flavin prosthetic group (probably not identical either with FAD or FMN), and the relationship of the cytochrome reductase to diaphorase will be discussed.

Transport of insoluble matter in nature: dependence upon complex formation. I. MANDL,* A. GRAUER* AND C. NEUBERG. *Dept. of Chemistry, Polytechnic Inst. of Brooklyn, Brooklyn, N. Y.*

In previous publications (*Arch. Biochem.* 19: 149, 1948; 20: 185, 1948; 23: 499, 1949) the authors drew attention to the property of important metabolites to form complexes with and hold in solution inorganic and organic compounds. It has now been found that this phenomenon applies to a much larger variety of substances than originally assumed and that almost all ionic reactions can be prevented by solubilizing agents which are omnicellular and known to play a part in vital biochemical processes. In view of the infinitely large number of different complexes that can be formed by the many functional groups of such compounds as nucleates, nucleotides, ATP, uronides, salts of hydroxy-acids, sugar phosphates, etc. an entirely new concept of the function of these substances as carriers is proposed. Insoluble metal salts, also cell poisons or drugs can be brought into solution, transported and finally reprecipitated by enzymatic decomposition of the 'solvent'. That this is possible was proved experimentally by dissolving such salts as e.g. calcium phosphate in sodium nucleate and reprecipitating by enzymes acting on the nucleate, similarly compounds dissolved in salts of conjugated glucuronic acid can be precipitated by β-glucuronidase. All these reactions, including the 'solubilization' take place under physiological conditions of pH, temperature etc. and continuously. The mechanism would apply equally well to similar processes taking place in the soil.

Radioactive lipids from tissue extracts of rats injected with $NaHC^{14}O_3$. LAWRENCE M. MARSHALL* AND FELIX FRIEDBERG. *Dept. of Biochemistry, Howard Univ. College of Medicine, Washington, D. C.*

Acetone extracts of liver and brain of rats sacrificed 1.5 and 4 hours after intraperitoneal injection with $NaHC^{14}O_3$ were chromatographed on silica gel columns with chloroform-amyl alcohol solvents. When radioactivity/gram of tissue was plotted against fractions of the effluent, a peak preceding those of acetate and fumarate showed a higher total activity than any other single fraction of the chromatogram. Elementary analysis of this fraction showed 74.3% carbon and 13.9% hydrogen. Solutions of the fraction in carbon disulfide and chloroform absorbed at 3000 cm^{-1} in a manner typical of a fatty acid showing hydrogen bonding and at 1700 cm^{-1} in a way characteristic of a mixture of fatty acid and fatty acid ester (*Anal. Chem.* 22: 1498, 1950). The infrared spectrum between 4000 cm^{-1} and 700 cm^{-1} for these lipids obtained from liver was similar but not identical to that of the lipids isolated from brain. The ratios of total radioactivity of the lipid fraction to total radioactivity for combined organic acids appearing on the chromatogram between lactate and aconitate were nearly identical for extracts of brain obtained 1.5 and 4 hours after the administration of the labeled bicarbonate. This ratio was greater for extracts of liver at 4 hours than at 1.5 hours after injection.

Modification of hypercholesteremia by phosphorylated hesperidin. GUSTAV J. MARTIN AND J. M. BEILER.* *Research Labs., Natl. Drug Co., Philadelphia, Pa.*

Studies of atheromatosis have in general considered blood levels of cholesterol, 'giant molecules', and the degree and incidence of plaque development; all of which are correlated (A.

KEYS, *J. A. M. A.* 147: 1514, 1951). One phase not generally studied is that of permeability of intestinal mucosa as related to cholesterol absorption. Under the assumption that hyaluronidase would play a role in permeability, phosphorylated hesperidin which is a powerful inhibitor of this enzyme (J. M. BEILER AND G. J. MARTIN, *J. Biol. Chem.* 174: 31, 1948) was studied for its effect on cholesterol blood levels in the rabbit. Rabbits (12 in number) were fed a diet consisting of rabbit chow containing 1.5% peanut oil and 0.5% cholesterol. The blood cholesterol levels were determined prior to treatment and cholesterol diet. Then the group was placed on the cholesterol diet and subdivided so that half were given phosphorylated hesperidin for the first 10 days and the others were not. At the end of the 10-day period the groups were switched and cholesterol levels taken after another week. The results show a definite effect of the phosphorylated hesperidin in preventing the development of cholesteremia. For the first 10-day period the control group showed an average rise in blood cholesterol of 532 mg. %; the group getting phosphorylated hesperidin 285 mg. %. When the treatment was reversed during the second 7-day period, the control group showed an average rise of 128 mg. %; the group getting phosphorylated hesperidin an average drop of 140 mg. %. While the cholesteremia was not completely prevented, its development was attenuated and slowed.

Renal effects of prolonged feeding of cholesterol and bile salt in the rat. W. MARX, E. JAMESON,* H. A. EDMONDSON,* L. I. RICE* AND E. RIDENHOUR.* *Dept. of Biochemistry and Nutrition, Univ. of Southern California School of Medicine, Los Angeles.*

Electrophoretic studies were made on the serum of rats fed a diet containing 1.5% cholesterol and 0.5% bile salt, for periods of 10 months or longer. The serum patterns resembled those occasionally found in cases of proteinuria, in that 2 albumin peaks and an unusually high gamma-globulin peak were observed at pH 7.4. Determination of urinary 'protein' according to the clinical method of Shevky and Stafford in similarly treated rats showed a marked proteinuria in some, but not in all of the animals. Histological examination of the kidneys revealed significant pathology in the animals with proteinuria. The most striking changes observed were protein casts in the lumen of the tubules which correlated roughly with the degree of proteinuria. No significant lesions were seen in the glomeruli to account for the proteinuria.

***Cypridina* luciferin.** H. S. MASON. *Dept. of Biology, Princeton Univ., Princeton, N. J.*

An extract of the luminous ostracod crustacean, *Cypridina hilgendorfii*, purified with respect to the oxygen-sensitive luminescent substrate, luciferin, according to Anderson's procedure (*J. Gen. Physiol.* 19: 301, 1935), was chromatographed on paper in an atmosphere of hydrogen, using a variety of solvents. The preparation contained only 2 substances, both of which luminesced in the presence of luciferase and are accordingly called α- and β-luciferin. These substances did not fluoresce, and did not contain phosphorus or the carbonyl or phenolic groups. It was thus not possible to confirm any hypothesis previously advanced for the structure of luciferin. On the other hand the absorption spectrum of the purified concentrate (CHASE AND BRIGHAM, *J. Biol. Chem.* 190: 529, 1951) and the presence in it of nitrogen (GIESE AND CHASE, *J. Cell. & Comp. Physiol.* 16: 237, 1940) have been confirmed. The nitrogen has been shown to be in the form of a primary aliphatic amino group not associated with amino acid and luciferin has been further characterized by infrared spectrum and by elementary analysis.

Structure of a catechol and a 3,4-dihydroxyphenylalanine melanin. H. S. MASON AND A. LADA.* *Dept. of Biology, Princeton Univ., Princeton, N. J., and the U. S. Public Health Service, Bethesda, Md.*

Melanins prepared by oxidizing catechol at pH 5.1 and 3,4-dihydroxyphenylalanine at pH 6.8 in the presence of optimal quantities of purified mushroom tyrosinase, required 2.6 and 4.5 atoms of oxygen respectively per molecule of diphenol melanized. The purified products were examined with respect to elementary composition, diffraction of X-rays, binding of water, reducibility, infrared absorption, and base-binding as a function of pH. The properties of the catechol melanin are best expressed in terms of a polymer of *o*-benzoquinone containing quinonoid and hydroquinonoid units attached ring-to-ring through the 4 and 5 positions, and other structures arising from autoxidative hydroxylation and degradation of the principal units. Dihydroxyphenylalanine melanin appears to be a polymer of indole-5,6-quinone containing quinonoid and hydroquinonoid units; an average of 3 bonds join each unit of polymer to adjacent, coplanar, units, probably through couplings at the 3, 4, and 7 positions of the indole quinone system followed by internal condensations and secondary cross-linkings involving the 2 position. Some protein was bound during melanin formation, presumably by the quinone-amino or quinone-thiol reactions.

Metabolism of tryptophan by riboflavin-deficient rats. MERLE MASON (introduced by HOWARD B. LEWIS). *Dept. of Biological Chemistry, Univ. of Michigan Med. School, Ann Arbor.*

After anthranilic acid is fed to white rats,

fluorescent substances can be detected in the urine by paper chromatography. Six or 7 spots of different sizes and intensities are observed, the most intense of which appears to be due to the glucuronide of anthranilic acid. Eluates of this spot have an ultraviolet spectrum like that of anthranilic acid and, following hydrolysis with 0.2 N HCl, contain substances that migrate on paper-like anthranilic acid and glucuronic acid. The other fluorescent derivatives were not investigated. A small amount of anthranilic acid and considerable amounts of several of its fluorescent derivatives, including the glucuronide, are excreted by rats that are fed L-tryptophan after being deprived of riboflavin for 3–5 weeks. Under the same conditions, non-deficient rats excreted minute amounts of the glucuronide only. Evidence was obtained that anthranilic acid and its glucuronide are also formed from L-kynurenine by rat liver slices and that the glucuronide is formed from anthranilic acid by both liver and kidney slices. Liver slices from the deficient rats converted kynurenine to anthranilic acid and its glucuronide twice as rapidly as liver slices from the non-deficient rats.

Molecular weight and viscosity of sodium chondroitin sulfate. MARTIN B. MATHEWS (introduced by ALBERT DORFMAN). *Dept. of Pediatrics, Univ. of Chicago, Chicago, Ill.*

The molecular weight of native sodium chondroitin sulfate (CS) from mammalian cartilage has been reported as 30,000 (by end group titration) and 260,000 (by birefringence of flow). The discrepancy is due, in part, to difficulties in obtaining pure undegraded CS, and, in part, to methodological differences between molecular weight procedures. It appeared desirable to determine the molecular weight of preparations of pure CS by a method of thermodynamic validity and to correlate the values obtained with intrinsic viscosities. $Co(NH_3)_6Cl_3$ was utilized as a precipitation agent for CS in the purification of this material extracted by different methods. The purity of preparations was established by chemical analyses and electrophoresis. An incompletely purified preparation showed a highly viscous component which was a complex of protein and CS. The molecular weights of the preparations of CS were determined by an osmometric method. The values obtained are as follows: for strong alkali extracted material; av. M_N = 15,000, av. $[\eta]$ = 0.54; for KCl extracted material (from stored cartilage, J. EINBINDER AND M. SCHUBERT, *J. Biol. Chem.* 185, 725, 1950); av. M_N = 24,000, av. $[\eta]$ = 0.89; for $CaCl_2$ extracted material; M_N = 33,500, $[\eta]$ = 1.03; M_N = 43,300, $[\eta]$ = 1.10. The viscosity of CS in salt solutions is a function of concentration and charge of the cation. CS shows viscosity functions in pure water typical of linear polyelectrolytes and data conform to relation $\eta\ sp/C = A/(1 + BC^{\frac{1}{2}}) + D$ (R. M. FUOSS, *J. Polymer Sci.* 3: 603, 1948). (Supported by a grant from the Chicago Heart Association.)

Purification and properties of alkaline phosphatase from swine kidneys. JAMES C. MATHIES AND ELEANORE D. GOODMAN (introduced by O. H. GAEBLER). *Edsel B. Ford Inst. for Med. Research, Henry Ford Hosp., Detroit, Mich.*

Previous attempts (*Biochim. et Biophys. Acta* 7: 387, 1951; *Science*, in press, 1952) to purify the alkaline phosphatase of swine kidneys have been continued. The following additional methods have been studied: *1*) removal of tryptic contamination by means of kaolin adsorption; *2*) precipitation of the enzyme by means of titration to pH 4.75 with acetic acid, *3*) adsorption of impurities on tricalcium phosphate gel, *4*) precipitation of impurities by treatment with protamine, and *5*) the application of electrophoresis-convection. The most active enzyme preparation obtained at the time of presentation of this abstract, had a specific activity of 1020 phosphatase units/mg. of total nitrogen, which appears to be the purest material obtained from this source at this time. (The enzyme unit is defined as that amount of enzyme which will liberate 1 mg. of phenol from disodium phenyl phosphate in 15 minutes and at 25° C. under optimal conditions of pH and prior alanine activation.) Properties of the enzyme will also be discussed.

Selenium toxicity in rats as influenced by choline, betaine and methionine. KENNETH P. MCCONNELL (introduced by B. M. HENDRIX). *Dept. of Biochemistry and Nutrition, Univ. of Texas Med. Branch, Galveston.*

The present experiments were undertaken to examine the protective effect of choline, betaine and methionine on the toxic action of sodium selenite in the rat. The basal diet contained 18% casein, 0.3% cystine, 64% sucrose, 8% fat and adequate amounts of minerals and all the known vitamins including B_{12} and folic acid. Five groups of weanling male rats were fed the basal diet plus one of the following: *A*, 0.4% choline and 17.5 ppm Se; *B*, 0.4% betaine and 17.5 ppm Se; *C*, 0.4% methionine and 17.5 ppm Se; *D*, 17.5 ppm Se and no supplement; *E*, no supplement or selenium. The growth of the rats on the various diets was observed over a period of 60 days at which time all the rats in *group D* died. It was found that the rats receiving selenium grew less than the controls in *group E* and that there were no marked differences in body weights until the 40th day. After the 40th day the rats in *group D* failed to gain while the rats receiving choline,

betaine and methionine continued to grow. The survival of selenium-fed rats on the various supplements was greater than that of the group receiving no supplement. It is concluded from the data obtained that methyl supplements, whether choline, betaine or methionine afford partial but not complete protection against the toxic effects of selenium.

Immobilization of ATP in the bioluminescent reaction. WILLIAM D. MCELROY AND JANE COULOMBRE.* *McCollum-Pratt Inst., Johns Hopkins Univ., Baltimore, Md.*

When adenosine triphosphate is added to a non-luminous aqueous extract of firefly lanterns, a flash of light is emitted, which rapidly decays to a low steady state intensity. Evidence has been presented previously which suggests that most of the ATP is still present but, by some unknown reaction, has been made unavailable for maximum light production. The luciferin and luciferase have been separated and partially purified. With these preparations, it is possible to show that luciferin, as well as magnesium, is essential for the immobilization of ATP. Calcium inhibits the rate of light intensity decay resulting in a greater total light emitted. The results indicate that the immobilization of ATP is more sensitive to calcium than is the initial light reaction. However, compounds which compete with luciferin in the initial light reaction, such as benzimidazole, benzothiazole and benzotriazole, do not influence the rate of immobilization of ATP. The results will be discussed in relation to a proposed scheme for bioluminescence in firefly extracts.

Effects of x-irradiation on liver glycogen and blood sugar. RALPH W. MCKEE. *Cancer Research Inst. of the New England Deaconess Hosp. and Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.*

The fasting of albino rats immediately following total body x-radiation (1500r) resulted in what appeared to be a retarded rate of glycogenolysis. Even after 24–36 hours of fasting, liver glycogen values were 1.0–2.5% as compared with 0.1–0.2% in the control groups. To test the ability of the x-radiated rats to form liver glycogen, animals were fasted 20 hours prior to irradiating and then the fast continued 12 hours. In striking contrast to normal fasting animals, the x-radiated rats rapidly produced liver glycogen with values up to 1.5%. During fasting the blood sugar of irradiated rats falls less than control animals. The differences are more marked when glucose is administered. Likewise in x-radiated rabbits the blood sugar tolerance curves remain elevated for a longer time than normal. The curves in both species are not of a diabetic type and there appears to be no observable damage to the pancreas. Also increased activity of transaminases in liver tissue following animal radiation may have some relationship to this phenomenon. It is likely that this production of glycogen is a gluconeogenesis stimulated by the pituitary and the adrenals. *In vitro* studies with liver slices measuring glycogen synthesis from carbohydrates and amino acids as substrates, are being carried out.

Isolation of a polyglycerol phosphatide from dog liver. JOHN M. MCKIBBIN AND WANDA E. TAYLOR.* *Dept. of Biochemistry, State Univ. of New York Med. Center, Syracuse.*

Livers from freshly sacrificed dogs were frozen on dry ice, crushed and extracted with alcohol ether and the residues extracted again with $CHCl_3$ in Soxhlet extractors. The combined extracts in $CHCl_3$ solution were purified by repeatedly emulsifying in .25 M aqueous $MgCl_2$. The phosphatides were prepared by acetone precipitation and these treated with 95% ethanol in a deep freeze cabinet. The alcohol insoluble phosphatides in $CHCl_3$ solution were then placed on a silicic acid-celite column. The column was washed with a solution of 2% by volume of methanol in $CHCl_3$ and then eluted with 11% methanol in $CHCl_3$. The first eluted material was then dissolved in toluene and methanol added. The supernatant fraction was placed on a silicic acid-celite column and was eluted successively with 3.25%, 6%, 9%, 12%, 20% and 80% methanol in $CHCl_3$ respectively. These eluates have marked differences in composition. The 6% eluate contains the polyglycerol phosphatide in almost pure state. Inositol and amino N phosphatides are concentrated in the 12, 20 and 80% eluates whereas sphingolipids are concentrated in the 9 and 12% eluates. The unknown N containing lipids are concentrated in the 9 and 80% eluates. The polyglycerol phosphatide thus separated contains 3.79% P and 0.122% N. The molar N:P of .072 indicates that the nitrogenous lipid is a contaminant. The phosphatide contains fatty acid and glycerol in the molar ratio to P of 3:3:2 respectively. Glycerophosphoric acid has been isolated from acid hydrolysates as well as a phosphate ester of glycerol combined with some other unidentified substance. An unidentified water soluble carboxylic acid also appears to be present in hydrolysates of the phosphatide.

Respiratory activity of human leukocytes in vitro. GORDON R. MCKINNEY, R. WAYNE RUNDLES AND SAMUEL P. MARTIN (introduced by FREDERICK BERNHEIM). *Dept. of Medicine, Duke Univ. School of Medicine, Durham, N. C.*

Leukocytes were separated by gravity sedimentation with dextran (C. P. Emerson) from the blood of healthy individuals after it had been passed through a decalcifying resin and handled

without exposure to wettable surfaces. After 60 minutes' sedimentation at 4° C., the leukocytes were concentrated by centrifuging 15 minutes at 50 times gravity and decanting the supernatant plasma. The white cells were resuspended in plasma and Hanks' solution, modified to contain no divalent cations. The Q_{O_2} of these cells, determined by Warburg's direct method and reported as microliters $O_2/10^7$ cells/hour, was 6.3, a value which did not change upon the addition of citrate, *alpha*-ketoglutarate, succinate, fumarate, malate, oxalacetate or glutamate in final concentration of 0.01 M. Exogenous cytochrome *c* (0.00005 M) did not stimulate nor did malonate (0.01 M) inhibit respiration with or without added succinate. KCN and *p*-chloromercuribenzoate in a final concentration of 0.0001 M markedly depressed respiration, but 0.001 M DNP was without effect. When cells were broken by grinding with alumina gel, however, stimulation with succinate, *alpha*-ketoglutarate, oxalacetate, cytochrome *c*, and DNP, and inhibition with malonate were observed. This indicates the existence of succinoxidase and an active Krebs cycle in mature leukocytes. It was evident that an intact cellular membrane prevented the usual stimulation by Krebs cycle intermediates and the inhibition by malonate.

Irradiation-induced destruction of essential fatty acids and other metabolites. JAMES F. MEAD (introduced by WENDELL H. GRIFFITH). *Atomic Energy Project, Univ. of California School of Medicine, Los Angeles.*

The effect of x-irradiation on dilute aqueous solutions of the essential fatty acids (EFA) at pH 8–9 or dilute aqueous suspensions of their methyl esters has been found to be the initiation of a chain reaction which results in the production of free radicals containing conjugated double bond systems, and in the accumulation of peroxides. Very high yields of these materials are obtained by increasing the concentration of EFA above certain concentrations (5×10^{-3} M for linoleic) and by decreasing the rate of x-irradiation (for example, to 10r/min.). Other materials, such as vitamin A, which are not particularly susceptible to low doses of x-irradiation when present as the sole solute, are rapidly destroyed when irradiated in solutions or emulsions containing EFA. This destruction is probably brought about both by the free radicals and the peroxides formed from the EFA, and the free radical destruction results in a partial protection of the EFA from the effects of irradiation. The mechanism is discussed in terms of a possible model for the biochemical effect or irradiation on animals.

Nutritional factors in lipogenesis from acetate in tissue slices of the rat. GRACE MEDES AND SIDNEY WEINHOUSE. *Lankenau Hosp. Research Inst. and Inst. for Cancer Research, Philadelphia, Pa.*

Incorporation of acetate carbon into fatty acids of liver slices from normal, well-nourished rats, studied with C^{14}-methyl-labeled acetate, was found to proceed linearly for at least 4 hours *in vitro*. Lipogenesis from acetate was greatly impaired in liver slices from rats fasted or chronically undernourished, but highest rates of acetate incorporation were observed when the animals were refed for 24 hours following varying periods of fasting or food restriction. Lipogenesis occurred in kidney, heart, spleen and testis slices, but at considerably lower rates than were observed for liver. With neither liver nor extrahepatic tissues was there any correlation between the incorporation of acetate carbon into fatty acids and its oxidation to CO_2. However, under all conditions acetate oxidation was considerably higher than its incorporation into fatty acids. Lipogenesis from acetate was equally diminished in thiamin-deficient rats and their pair-fed controls. In thiamin-deficient rats neither food nor thiamin alone restored fatty acid synthesis; however, administration of both food and thiamin resulted in prompt resumption of normal or above normal lipogenesis from acetate. In contrast with 'nourished' liver slices, 'fasted' slices were unresponsive to the addition of insulin. It is concluded that the defect in fatty acid synthesis during undernourishment occurs somewhere in the process of conversion of acetate to fatty acid, and that this step is not closely related metabolically to acetate oxidation.

Cellular microspectroscopy of hemoglobin-derived pigments. ROBERT C. MELLORS. *Memorial Center for Cancer and Allied Diseases, New York City.*

By means of a universal microscope and microspectrometer that permits a recording of the light absorption of microscopic objects over the entire optical spectrum (wavelength region, 0.2–15μ), an attempt is being made to characterize the absorption spectra of hemoglobin-derived pigments that occur normally and abnormally in tissue cells. The analysis is applied to submicrogram quantities (order of 10^{-6} μg.) of pure materials such as the hemoglobins, hematin, bile pigments and the iron-proteins, ferritin and hemosiderin; to oxidation, reduction and metallic derivatives of certain of these compounds; and to pigments as they occur in individual tissue cells, particularly under circumstances in which there is a preponderant localization of one or another substance. Microabsorption spectra in the ultraviolet and the visible regions (wavelength region, 0.3–0.8μ) on tissue sections, with a sample of

thickness, 10μ, and of cross-sectional area, 4μ^2, are recorded for the hemoglobins of the red blood cell; the bile pigments in the liver cell; and the iron-protein, hemosiderin, in cells of the reticuloendothelial system.

Metabolism of the perfused cat brain in vivo. HERBERT L. MELTZER,* REGINALD M. TAYLOR* AND WARREN M. SPERRY. *Depts. of Biochemistry and Experimental Psychiatry, New York State Psychiatric Inst., and Depts. of Biochemistry and Psychiatry, Columbia Univ., New York City.*

Some aspects of the metabolic requirements of the perfused cat brain, isolated *in vivo* according to the Geiger-Magnes technique (*Am. J. Physiol.* 149: 517, 1947), were investigated. Acetate 1-C^{14}, pyruvamide 2-C^{14}, and lactate 2-C^{14} were found to give rise to labeled CO_2. Labeled glucose was isolated from brain tissue following perfusion with pyruvamide. The requirement of the perfused brain for glucose was found to be markedly reduced if lactate was present in the perfusion fluid. Under such conditions the glucose concentration of the brain was found to be as low as 10 mg/100 gm., although the functional activity, as measured by the electrocorticogram and corneal reflexes, was about the same as was observed when the brain was perfused with a fluid containing large amounts of glucose. (This investigation was supported by a research grant from the National Institutes of Health, Public Health Service.)

Structure and microbiological activity of some dinucleotides isolated from RNA. R. B. MERRIFIELD* AND D. W. WOOLLEY. *Rockefeller Inst. for Med. Research, New York City.*

Lactobacillus helveticus 335 has previously been shown to require uracil for growth and to be unable to substitute other pyrimidines, purines, nucleosides or mononucleotides for this purpose. When yeast ribonucleic acid was treated with 6*N* HCl for 3 minutes at 25° the product completely replaced uracil, even though it contained no free uracil. Fractionation of this partial hydrolysate on ion-exchange resin has yielded analytically pure dinucleotides possessing microbiological activity approximately equal to uracil on a molar basis. The structure of a guanylic-cytidylic dinucleotide was demonstrated in the following manner to be 5′ (a (or b) guanylyl) cytidylic acid b. Alkaline hydrolysis gave guanylic acids a and b and cytidylic acid b. Intestinal alkaline phosphatase, partially inhibited by arsenate, yielded guanosine, cytidine and cytidine-5′-phosphate. The latter was identified on paper, on ion-exchange columns and by Hepple's 5′-phosphatase. Schmidt's prostate acid phosphatase liberated one mole of inorganic phosphate from the dinucleotide, and the isolated product, containing one mole of phosphorus, yielded guanylic acids a and b, and cytidine on alkaline hydrolysis. The dinucleotide was unaffected by ribonuclease or by 5′-phosphatase. By similar procedures the structures of the dinucleotides adenylic-cytidylic, uridylic-cytidylic, cytidylic-cytidylic, and adenylic-guanylic were studied. Those compounds containing cytidine-5′-phosphate esterified with either a pyrimidine or a purine riboside were microbiologically active, while those not containing this grouping were not. These facts suggest that the nucleosides and monocucleotides are not along the main pathway of uracil utilization.

Role of vitamin B_6 in deamination of serine. DAVID E. METZLER* AND ESMOND E. SNELL. *Dept. of Chemistry, Univ. of Texas, Austin.*

Pyridoxal (1–10mM) and aluminum or copper salts (1 mM) cause the rapid deamination of serine (10 mM) at 100° and pH 4–5. Pyruvate and ammonia are produced almost quantitatively; pyridoxal concentration changes very little. Cysteine yields ammonia, pyruvate and H_2S. This suggested that serine deaminase, like cysteine desulfurase, might be a vitamin B_6 enzyme. A B_6 requiring mutant of *E. coli* could be grown with or without B_6 on a 'complete' synthetic medium. Cells grown 16 hours were washed, suspended in 0.1 M phosphate buffer, pH 7, and shaken with toluene or vacuum dried. Their capacity to deaminate DL-serine was determined by measuring pyruvate production. With B_6-deficient cells, activity was increased 50–100% by addition of 0.05 mM pyridoxal phosphate; cells grown with B_6 gained only 10–20%. Cell-free extracts prepared at pH 7–7.8 from these or from more active cells grown on tryptone medium gradually lost activity which could be restored by pyridoxal phosphate. Pyridoxal phosphate caused a 10-fold increase in activity of such extracts from B_6-deficient cells. With extracts the pH optimum was about 8.2. No activation by adenylic acid, glutathione, or metal ions was detected; threonine was deaminated very slowly. As reported by Wood and Gunsalus, (*J. Biol. Chem.* 181, 171, 1949), cells dried at pH 7.8 with glutathione are activated by adenylic acid and rapidly attack threonine. Activity of such cells from tryptone medium is only slightly increased by pyridoxal phosphate. Differences in the two deaminase preparations are being studied.

Pasteur effect in yeast lacking cytochrome oxidase. OTTO MEYERHOF[1] AND HAROLD M. DAVIDSON.* *Dept. of Physiological Chemistry, Univ. of Pennsylvania School of Medicine, Philadelphia, Pa.*

Respiration and fermentation were studied manometrically in mutant yeast of Ephrussi lack-

[1] Deceased Oct. 7, 1951.

ing cytochrome oxidase. Respiration occurred only when fermentable sugar was added and then only in 100% oxygen. However in quick-dried preparations of the mutant of brewer's yeast the oxidation of reduced DPN was observed in the absence of added sugar. The respiration, insensitive to cyanide, was temporarily accelerated during the early stages of inhibition of fermentation by iodoacetate, possibly due to the transient accumulation of reduced DPN. Iodoacetate also induced respiration in air. Fermentation was inhibited aerobically when the tension of oxygen was great enough to permit respiration. The Pasteur effect was conventional in that aerobic fermentation was steady and the higher anaerobic rate was resumed after oxygen was replaced by nitrogen; but it was unconventional because the onset and reversibility of the oxidative inhibition of fermentation was prolonged, not instantaneous, and because the Meyerhof quotient was 8–15 instead of 1–2. No evidence was adduced that hydrogen peroxide could be held responsible for the aerobic inhibition of fermentation. The results were consistent with the view that the mutant respires via flavine enzymes. With this organism at least the hypothesis cannot be valid that the oxidation-reduction state of cytochrome oxidase determines the Pasteur effect. (Aided by a grant from the Dazian Foundation for Medical Research.)

Reaction of diisopropyl fluorophosphate (DFP) with red blood cell cholinesterase. HARRY O. MICHEL. *Enzyme Chemistry Branch, Chemical Corps Med. Labs., Army Chemical Center, Md.*

The relationship between DFP concentration and cholinesterase inactivation was investigated using red cells from human and beef blood and partially purified preparations of beef red cell cholinesterase. A large fraction of added DFP was found to react preferentially with components in red cells other than cholinesterase. DFP labeled with P^{32}, when added to whole red cells, was rapidly bound to material precipitable by 5% trichloroacetic acid. Of the total DFP bound/ml. of red cells when cholinesterase inactivation is complete, about 95% was bound before any appreciable inactivation of the enzyme was observed. Labeled DFP, when allowed to react with a highly purified preparation of beef red cell cholinesterase obtained commercially, was bound in amounts proportional to enzyme inactivation. Approximately twice as much DFP was bound per unit of enzyme activity by this preparation of beef red cell cholinesterase as was found previously for electric eel cholinesterase. The amount of cholinesterase in human red cells, assuming that one mole of enzyme is inactivated by one mole of DFP, is estimated to be equivalent to a concentration of 4×10^{-8} M.

Binding of ions to myosin. E. MIHALYI AND B. N. GHOSH (introduced by JAMES H. JONES). *Inst. for Muscle Research, Marine Biological Lab., Woods Hole, Mass.*

The interaction of myosin with ions was investigated: *a*) by equilibrium dialysis and *b*) by the change of pH of a salt-free myosin solution upon salt addition. The dialysis method was used with methyl orange and thiocyanate ions. At pH 6.8 and ionic strength 0.3 the binding of methyl orange is slight, corresponding to the calculated 'binding index' of 2.4. Thiocyanate is practically not bound above the isoionic point but becomes increasingly bound as the pH is lowered. Temperature does not affect the extent of binding. Chloride ions compete strongly with the thiocyanate ions. The shift of pH of a salt-free myosin solution acid to the isoionic point shows a strong interaction between myosin and anions. The effectiveness of the investigated anions decreases in the following order: F, SCN, I, Br, Cl, SO_4. The effect of the strongly bound F and SCN is apparent, though less pronounced, even above the isoionic point, while the others are ineffective in this region. Divalent cations seem to be bound above, as well as to a smaller extent, below the isoionic point. (This work was supported by the Armour Research Laboratories.)

Histochemical characterization of articular cartilages of poliomyelitis patients. JAMES S. MILES,* LILLIAN EICHELBERGER AND MICHAEL ROMA.* *Dept. of Surgery, Univ. of Chicago, Chicago, Ill.*

Chemical data will be presented for the values of hyaline cartilages removed from the joint surfaces of poliomyelitis patients during reconstructive procedures. These data are utilized to describe *1*) the percentages of extracellular and intracellular solids in 100 grams of the total cartilage solids, *2*) the percentages of connective tissue solids and chondroitin sulfate solids in 100 grams of extracellular solids and *3*) the histochemical characterization of the extra- and intracellular phases per kg. of fresh cartilage. From the acquired data it seemed reasonable to ascribe approximately 75% of the cartilage solids of these patients to the extracellular solids and 25% to the cellular solids. This generalization does not vary significantly from the values obtained on normal puppy articular cartilages. Great differences are noted however when the extracellular solids are considered from the standpoint of their separate parts: namely the connective tissue solids, varying between 69 and 92% and the chondroitin sulfate solids varying between 8 and 31%. The connective tissue solids were found to be a larger component than the chondroitin sulfate solids. Furthermore, the connective tissue solids varied inversely with the chondroitin sul-

fate solids. The histochemical patterns for the masses of the extra- and intracellular phases per kg. of fresh cartilage will be presented and discussed. The relative mass of the solids of the cells (chondrocytes) per kg. of cartilage varied between 4 and 8%. The calculated percentages of cellular water in many patients were low, or a state of dehydration of the cells existed.

Thermal stability of a pyruvic oxidase. WALTER MILITZER AND LOUISE BURNS.* *Dept. of Chemistry, Univ. of Nebraska, Lincoln.*

A pyruvic oxidase was found in the cell granule isolated from a thermophilic bacterium (isolate no. 2184). The enzyme system was dependent on magnesium and diphosphothiamine. Fluoride inhibited completely. The enzyme system can function in borate buffer (pH 6.5) without phosphate, although activity was greater when phosphate was present. Assays were conducted at 45°C. by following oxygen consumption. Unlike other enzymes isolated and identified from this organism (*Arch. Biochem. Biophys.* 31: 416, 1951; 26: 299, 1950), pyruvic oxidase did not resist inactivation at the growing temperature of the organism (65° C.). A treatment at 60°C. for 1 hour completely destroyed the pyruvic oxidase activity of the cell granule but did not injure enzymes such as cytochrome oxidase, apyrase, succinic oxidase nor cytochrome *b*. Magnesium added to the suspension of the cell granules during heating only partly protected the enzyme against inactivation and could not account for the stability of the enzyme system in the growing cells. Pyruvic oxidase in the intact cell withstood heating for an hour at 65°C. When, however, the cells were broken up with lysozyme, the enzyme did not retain its ability to resist destruction. Factors responsible for this anomaly have been studied.

Myosin preparations from the rabbit, rat and mouse. GAIL LORENZ MILLER, RICHARD H. GOLDER,* EDWARD S. EITELMAN* AND ELIZABETH ESHELMAN MILLER.* *Inst. for Cancer Research and the Lankenau Hosp. Research Inst., Philadelphia, Pa.*

Muscle tissue was ground in a mortar cooled with solid carbon dioxide and was extracted at 2°C. for 5 minutes with 0.6 ionic strength pH 6.5 buffered potassium chloride solution. The mixture was clarified by centrifugation and the myosin was precipitated from the supernatant fluid by a 15-fold dilution with water. The precipitated myosin was finally redissolved in a medium identical with that used for extraction. The yield of myosin thus obtained was more reproducible and one-third higher than that obtained when dry ice was added to the tissue during the grinding or when fresh muscle ground in a Latapie mill was extracted. Electrophoretic analysis of the myosin revealed the presence of a slowly migrating impurity, while ultracentrifugal analysis revealed a rapidly sedimenting impurity. Further studies are being made on the identification of the impurities and their possible correspondence to one another. Preparations of myosin isolated from muscles of the rabbit, rat or mouse were not readily distinguishable on the basis of yield, mobility or sedimentation rate. However, to judge from the differences between sedimentation patterns obtained at room and refrigerator temperatures, the mouse myosin appears to be much less stable than the others.

Factors affecting biosynthesis of plasma albumin and globulin fractions as studied with the aid of lysine-ε-C^{14} and histidine-2-C^{14}. LEON L. MILLER AND WM. F. BALE.* *Dept. of Radiation Biology, Univ. of Rochester, School of Medicine and Dentistry, N. Y.*

Lysine-ε-C^{14} and histidine-2-C^{14} have been used in studies seeking to assess factors which determine the albumin to globulin ratio of the plasma proteins. Both intact rats and isolated perfused rat livers yield data which point strongly to the conclusion that the blood amino acid level is the factor conditioning the quantitative response of the liver in the biosynthesis of the albumin and globulin fractions. Where the amino acid level is comparatively high, the synthesis of the plasma albumin fraction is favored (high A/G). Where the amino acid level is low, the synthesis of the plasma globulin fraction is favored (low A/G). These observations make understandable variations in the A/G ratio observed in starvation, protein depletion, and liver disease with and without hepatic portal obstruction. Factors such as the blood glucose level, anoxia, and the A/G ratio of the circulating blood plasma, appear to be without significant direct effect on the albumin to globulin ratio of newly formed plasma proteins.

Hydrolysis of acetylated ovalbumin by crystalline pepsin. LILA MILLER AND OTTO NEUHAUS.* *Dept. of Biological Chemistry, Univ. of Michigan, Ann Arbor.*

The configuration assumed to be necessary for the action of pepsin has been based largely on experiments with synthetic peptides. Studies of the action of crystalline pepsin on purified proteins which have been subjected to the action of chemical agents are limited; in most cases the modified proteins have not been characterized or isolated. In the present study, crystalline ovalbumin was altered by acetylation with ketene. At pH 5.6, 68% of the amino groups were acetylated and at 9.5, the remaining amino groups and approximately 54% of the hydroxyphenyl groups. These modified proteins were dialyzed, precipitated with sodium sulfate, purified by dialysis

and the resultant product lyophilized. In the enzyme studies with these products, the average strength of the crystalline pepsin, expressed as hemoglobin units, was 4.1×10^{-3} units/mg. of substrate nitrogen. The course of the hydrolyses was followed by the determination of the amino nitrogen and the trichloroacetic acid precipitable nitrogen. The increases in amino nitrogen occurring during 24-hour incubation at 30° were 16, 14 and 6% of the total nitrogen with untreated, N-acetylated, and O,N-acetylated ovalbumins as the respective substrates. For the corresponding substrates the precipitable nitrogens in this period were 4, 12 and 60% of the substrate nitrogen respectively. The interpretation of these results will be discussed.

Enzymatic oxidation of propandiol phosphate. O. NEAL MILLER, C. G. HUGGINS, K. ARAI AND R. P. SANDMAN (introduced by D. BAILEY CALVIN). *Dept. of Biochemistry and Nutrition, Univ. of Texas Med. Branch, Galveston, Texas.*

Propandiol 1-phosphate (PDP) is a normal constituent of liver, kidney, and brain tissues, ranging from 1–5% of the acid-soluble phosphorus. Similarities between PDP and triose phosphates of the glycolytic scheme and to alpha-glycerophosphate are apparent. It was of interest to determine if alpha-glycerophosphate dehydrogenase would oxidize PDP. The compound has been synthesized by usual techniques and purified by adsorption on ion-exchange resins. The product is free from other isomers and starting materials. Although the phosphate of PDP is stable to long periods of boiling with half-normal alkali or normal acid, it is completely liberated with crude preparations of intestinal alkaline phosphatase in 40 minutes at 40° C. A crystalline preparation (from rabbit muscle) of myogen A (T. BARANOWSKI, *Z. physiol. Chem.* 260: 43, 1939) was found to oxidize PDP as well as alpha-glycerophosphate. Activity was determined in the Beckman spectrophotometer by measuring the appearance of DPN-2H in a reaction mixture containing DPN, substrate, and enzyme in a phosphate buffer (PH 8.0). The reaction with PDP and rabbit muscle enzyme reached equilibrium after 15 minutes; after 30 minutes a heart-muscle extract was added to the same cuvette and blank. Initial reaction rate was restored for 40 minutes suggesting the removal of the reaction products of PDP. Beta-glycerophosphate has little or no activity with this system. Myogen A is known to contain alpha-glycerophosphate dehydrogenase and aldolase. Electrophoretic studies on the crystalline enzyme to determine the presence of a possible third enzyme component will be reported.

Studies on Δ^{-7} cholestenol in skin. W. L. MILLER, JR.* AND C. A. BAUMANN. *Dept. of Biochemistry, Univ. of Wisconsin, Madison.*

When crude sterols from rat skin are treated with a modified Lieberman-Burchard reagent, an intense blue-green color forms immediately, whereas pure cholesterol or the sterol from most other animal sources forms the characteristic color only slowly. In this laboratory D. R. Idler has recently isolated the fast-acting component and identified it as Δ^{-7} cholestenol. The distribution of Δ^{-7} cholestenol has been studied by measuring the total fast-acting sterol in a series of digitonides, and correcting for the 7-dehydro cholesterol measured spectrophotometrically in the regenerated free sterols. The concentration of Δ^{-7} cholestenol ranged from 25–35% of the total sterol in rat skin. This proportion did not vary with sex, basal metabolic rate, nor in severe pantothenic acid deficiency, but it did increase with the age of the rat. Other organs contained only negligible amounts of fast-acting sterol. The skins of the cotton rat and of the wild rat were similar to those of the laboratory rat. Sterols from the skins of the mouse and guinea pig contained about 15% of the fast-acting component, and sheep skin also contained a measurable amount. Dog skin was variable, while the amounts of Δ^{-7} cholestenol appeared to be negligible in the skins of the pig, calf, cat, rabbit and man (skin over mammary). Δ^{-7} cholestenol was also determined by the reduction of selenious acid.

Formaldehyde-pyruvate condensing enzyme. MARGARET C. MITCHELL* AND CAMILLO ARTOM. *Dept. of Biochemistry, Bowman Gray School of Medicine, Winston-Salem, N. C.*

The widespread occurrence in animal tissue of a formaldehyde-pyruvate condensing enzyme has been reported (*Federation Proc.* 10: 224, 1951). We have studied further the properties of this enzyme in partially purified preparations ('large granules' of rat liver). Our previous data on some characteristics of the addition product(s) have been confirmed and extended by the use of C^{14}-labeled formaldehyde. It appears now that the enzyme is different from the diphosphothiamine-metal-proteins catalyzing the formation of acyloins from other aldehydes and pyruvate. Since in the reaction between formaldehyde and pyruvate, no CO_2 is evolved, it seems likely that the initial product is a four carbon compound from which other compounds can be formed enzymatically or non-enzymatically. (Aided by the U. S. Atomic Energy Commission, Contract No. AT-(40-1)-288, Title II).

Polymerization of actin. W. F. H. M. MOMMAERTS. *Dept. of Biochemistry, Duke Univ., School of Medicine, Durham, N. C.*

According to previous studies with pure actin, the polymerization of this protein entails the stoichiometric breakdown of ATP, according to the equation: actin + ATP = actin$_{(polym.)}$ + ADP + phosphate (*1*). In this reaction, the amount of actin which liberates 1 mole of phosphate equals the molecular weight, as determined by lightscattering. This equation holds in a variety of conditions. In particular, there is a concomitant effect upon both polymerization velocity and rate of phosphate liberation under the influence of experimental variables. This is strikingly demonstrated by the application of inhibitors like chloromercuric benzoate, urea and iodide. These reagents delay both the polymerization, and the liberation of phosphate, and block both processes at sufficiently high concentrations. It is, nevertheless, probable that *equation 1* is of a multiple nature, proceeding in two separate steps, the first of which is a phosphorylation of the protein: actin + ATP = actin-PH + ADP (*2a*); actin-PH + actin = actin-actin + phosphate (*2b*). There are indications that it is the sulfhydryl group of actin which interacts with the ATP, preliminary to the condensation reaction, in analogy with the behavior of coenzyme A. No phosphorylated actin has been found to appear in measurable concentration under a variety of circumstances.

Distribution of C^{14} in the rat after oral administration of a dioleylstearin 1-C^{14}. MARGARET G. MOREHOUSE AND MILLIE THOMPSON (introduced by JOHN W. MEHL). *Dept. of Biochemistry and Nutrition, Univ. of Southern California School of Medicine, Los Angeles.*

A triglyceride containing oleic and stearic acids has been synthesized by direct esterification. Physical constants show it to closely resemble a mixed dioleylstearin with a melting point of 35°C. Stearic acid 1-C^{14} was used to label the compound. Female rats weighing 175 ± 10 gm. were fasted 48 hours, fed 0.42 gm. of the fat, and sacrificed at intervals corresponding to early, median and late absorption periods previously determined by feeding an unlabelled fat. The distribution of activity was established by determining the amounts and specific activity of the material remaining in the digestive tract, of the phospholipid and neutral fat of the gut wall, of the same substances in the liver and of the ether soluble material after acidification of the saponified carcass. At the 3-hour period when half the fat has been absorbed activity determinations account for 88% of that administered with 40% of the activity appearing in the carcass lipids. In both intestine and liver the specific activity of the neutral fat is higher than that of the phospholipids. Results from studies on the other 2 periods will also be discussed with particular reference to the specific activity of the phospholipids and neutral fat of the intestinal wall. (Supported by a grant from Swift and Co.)

Adhesion of fibrin clots to tissue. PETER R. MORRISON AND FREDRIC DOPPELT.* *Depts. of Physiology and Zoology, Univ. of Wisconsin, Madison.*

Fibrin clots were formed from partly purified bovine Fraction I (3 gm. fibrinogen/l.) and thrombin (1 unit/cc.) and clotted for 20 minutes. Tissues were removed from freshly killed dogs and a thin square was stretched tightly over a metal rod by a plastic ring. The clot was formed between this surface and a cloth covered disc. The force necessary to separate the clot from the surface was then measured as previously described (MORRISON AND SEILER, *Am. J. Physiol.*, in press) but under saline to eliminate surface tension. Of the four tissues employed, artery, vein, peritoneum and muscle, adhesion to artery was the lowest (1 gm/cm.^{2}), this being, however, comparable to the best nonbiological surfaces tested. Adhesion to vein and peritoneum was better (2 gm/cm.^{2}) and adhesion to muscle (cut diaphragm) was best (3 gm/cm.^{2}). Platelets had a deleterious effect on vein, artery and peritoneum with half the normal concentration reducing adhesion to one third or less. On muscle, however, platelets increased adhesion up to two-fold. Red blood corpuscles (25 v.%) had no effect on adhesion to vein, peritoneum or muscle. In contrast, whole serum increased adhesion markedly, up to 3-fold on vein and peritoneum (7 gm/cm.^{2}) and up to 5-fold on muscle (15 gm/cm.^{2}). Calcium had no effect at concentrations of 10^{-4} or 10^{-3} M but at 10^{-2} M the adhesive force was doubled.

Activating influence of α-estradiol on the *in vitro* incorporation of glycine-2C^{14} into surviving rat uteri. GERALD C. MUELLER AND GLENN N. YANAGI (introduced by H. F. DEUTSCH). *McArdle Memorial Lab., Univ. of Wisconsin Med. School, Madison.*

The rate of glycine-2C^{14} incorporation into protein of surviving rat uteri was studied at frequent intervals throughout a 96-hour period after the intravenous administration of 10 μg. of α-estradiol. Within 4 hours all uteri responded with a significant increase in the rate of glycine incorporation, and at 20 hours a maximal rate of 2–3 times that of the control uteri was obtained. Subsequently this rate fell abruptly to the control level at 36 hours and then rose slightly to a plateau for the remainder of the 96-hour period. Variation in the level of α-estradiol indicated that a maximal rate of response was obtained with 10 μg. The incorporation was highly dependent on glucose and oxygen. Dinitrophenol .0001 M or NaCN .0001 M blocked the incorporation of glycine completely; however at lower concentrations of dinitrophenol the estrogen-treated uteri were inhibited less than

the control uteri. Isolation of the amino acids from the hydrolyzed protein showed that 90–95% of the activity was accounted for as glycine with only small amounts present in the serine fraction. The addition of .001 M serine to the reaction media significantly lowered the specific activity of activated uteri, but produced an increase in the controls. Formate .001 M has the opposite effect. Estradiol *in vitro* produced a slight activating effect in some cases. Of other steroids tested *in vitro* desoxycorticosterone and progesterone were found to be toxic to the glycine incorporation at low levels, where as cortisone had no effect. Additional data will be reported on other aspects of this system.

A study of the properties of native ACTH. PAUL L. MUNSON, ANNA MORSE* AND EDWARD MILEY.* *Biological Research Labs., Harvard School of Dental Medicine, Boston, Mass.*

Centrifugation of frozen hog pituitaries at 25,000 g and 1–5°C. yields 0.35 ml/gm. of a dark red exudate which contains ACTH, presumably in its native state, in high concentration. The exudate provides a convenient starting material for purification of native pituitary hormones without the use of protein denaturing agents. The frozen glands thaw during centrifugation and pack at the bottom of the tube, but this does not result in any gross distortion of structure. Histologically, the centrifuged glands are not noticeably different from non-centrifuged controls. Cytologically, the cells of the frozen glands are intact, but the nuclei appear dense and shrunken. The blood vessels appear to have been drained of their contents. The exudate exhibits a significant amount of ACTH activity at a dilution of 1:64,000 when assayed with hypophysectomized rats by the Munson modification of the method of Sayers *et al.* Biological assays of the exudate for other hypophyseal hormones and purification studies are in progress.

Partial purification and properties of brain phosphofructokinase. J. A. MUNTZ. *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

Phosphofructokinase (PFK) has been prepared from acetone powders of rat, dog and beef brain. Aqueous extracts of the acetone powders were heated at 62°C. for 5 minutes in the presence of 0.0015 M ATP and then frozen and thawed. Approximately half of the protein flocculated out of solution and was removed by centrifugation. All of the PFK activity remained in the supernatant. Most of the PFK could then be precipitated with $(NH_4)_2SO_4$ at pH 8 between 35 and 52% saturation and the activity was increased 4- to 5-fold per unit of protein as compared to the acetone powder extract. This preparation does not catalyze the phosphorylation of glucose, fructose, glucose-1-phosphate or glucose-6-phosphate and it contains negligible amounts of apyrase and myokinase. The enzyme is activated by Mn^{++}, Mg^{++}, and Co^{++}, the respective K_m values being 5.6×10^{-4}, 1.2×10^{-3}, and 1.8×10^{-3}. Zn^{++} and Ni^{++} have a small activating effect. NH_4^+ is required for full activity and the K_m is 4×10^{-4}, while K^+ has only a slight stimulatory effect. With the enzyme preparation, the reaction ATP + F-6-P → HDP + ADP, does not go to completion. This aspect of the problem is being investigated.

Excretion of formaldehydegenic substances by normal and scorbutic guinea pigs. ELI M. NADEL* AND JOHN J. SCHNEIDER. *Pathology Section, National Cancer Inst., Bethesda, Md., and Dept. of Exper. Medicine, Jefferson Med. College, Philadelphia, Pa.*

The excretion of formaldehydegenic (FG) substances by normal guinea pigs is high, being of the order of 100 gamma/24-hour period. Animals on a scorbutigenic diet showed first normal, then low, and as the deficiency disease progressed, very high FG titres (E. M. NADEL AND J. J. SCHNEIDER, *J. Clin. Endocrinol.*, 11: 791, 1951 abstract). After ascorbic acid therapy, animals with high titres excreted diminished amounts of FG substances. Starved animals given daily supplements of ascorbic acid had normal FG excretion values. Fractionation of urinary extracts from normal and scorbutic animals showed that the periodate-reacting material was largely water soluble and ketonic in nature. The purest fraction of extracts from urine of scorbutic animals showed an absorption maximum at 240 mμ in alcoholic solution. Sulfuric acid solutions of this fraction displayed a green fluorescence and absorption maxima at 280 mμ, 390 mμ, and 470 mμ, corresponding to those given by Zaffaroni (*J. Am. Chem. Soc.*, 72: 3828, 1950) for 17-hydroxy corticosterone. Experiments concerned with the *in vitro* metabolism of Compounds E and F by liver slices from normal, starved and scorbutic guinea pigs have been completed and will also be presented.

Effect of acetoacetate on insulin action. MARY E. NAGLER (introduced by H. C. TIDWELL). *Biochemistry Dept., Southwestern Med. School, Dallas, Texas.*

After extensive studies on the effect of ketone bodies on carbohydrate metabolism, Nath and co-workers believe that these bodies first produce a stimulating effect and finally exhaustion atrophy of the islet tissue. They suggest that this might be the result of either an inactivating action on the insulin or a direct effect on the insulin secreting mechanism. A disturbance of phosphorylation was also suggested as a reason for a decreased

glycogen storage in the muscle of animals given acetoacetate. Glycogen content, glucose and acetoacetate loss were measured when a surviving rat diaphragm was incubated in a phosphate buffer solution containing 200 mg.% glucose. The incubating medium contained either glucose; glucose and insulin (0.05 units/ml.); glucose, insulin and acetoacetate (50 mg.%); or glucose and acetoacetate. The glucose losses and glycogen content of tissues were the same when the medium contained glucose or glucose and acetoacetate. The values obtained in both mediums containing insulin were similar but they were significantly greater than those without insulin. The failure of acetoacetate to cause any decrease in glucose loss or glycogen formation, with the smallest quantity of insulin found effective, would indicate no inactivating effect on the insulin itself. Ketone losses were similar with and without insulin. The similar tissue glycogen formation in the presence of the insulin and ketone would not be consistent with a disturbed phosphorylation. Hence under these conditions any action of the ketone bodies must be explained by other means than insulin inactivation or a disturbed phosphorylation.

Glutamic acid decarboxylase from *E. coli*. VICTOR A. NAJJAR AND JEAN FISHER.* *Dept. of Pediatrics, Johns Hopkins Univ. School of Medicine, Baltimore, Md.*

A strain of *E. coli* was isolated that showed a high glutamic acid decarboxylase activity. Acetone powder was prepared by adding 9 volumes of acetone to a suspension of cells harvested from an 18-hour culture at 37°C. in 3% trypticase soy broth. One mg. of powder decarboxylates about 1 micromole of glutamic acid/minute, when the enzyme is saturated with substrate at pH 5.0 in acetate buffer 0.15 M. Its activity against arginine and lysine under these conditions was 10 and 16% as much. No other amino acid was attacked. Little or no glutaminase activity was detectable. Lysine and arginine decarboxylase activity was destroyed by incubating a 20 mg/ml. suspension of the powder in water for 72 hours at 55°C. or in 0.2 M acetate buffer pH 5.0 for 48 hours at 37°C. Glutamic acid decarboxylase activity is not affected by this treatment or by dialysis against distilled water for 48 hours. The Michaelis constant is about 0.0045 M, pH optimum 4.8–5.5. Activity is directly proportional to enzyme concentration at saturation. Maximum activity is attained after 8 hours of growth at 37°C. and is maintained after 24 hours. A 4-hour culture shows 50–80% of maximum activity. Purification of the enzyme with ammonium sulfate fractionation and other properties will be presented.

Effect of copper deficiency on isocitric dehydrogenase activity in tomato leaves. ALVIN NASON (introduced by E. V. MCCOLLUM). *McCollum-Pratt Inst., Johns Hopkins Univ., Baltimore, Md.*

Cell-free extracts of tomato leaves from plants grown in mineral nutrient solution deficient in each of the micronutrient elements showed a marked increase of TPN-isocitric dehydrogenase activity in copper-deficient material. The latter averaged an approximate 15-fold activity increase, measured by the increase in TPNH, over normal tissues expressed either on a protein or dry weight basis. The presence of an activator or inhibitor in copper-deficient and control extracts, respectively, was ruled out by mixing experiments. Although copper deficiency resulted in a significant lowering of ascorbic acid oxidase and polyphenol oxidase as well as changes in other enzymes, mixing experiments also eliminated the possibility that a block in the transfer of electrons from TPNH was responsible for its enhanced accumulation. The presence of Cu^{++} had no significant effect except at high concentrations, 10^{-3}M final concentration of Cu^{++}, resulting in 90% inhibition of the enzyme. The addition of Cu^{++} to the deficient media at control levels (.02 parts/million) gave a progressive decrease of the enzyme with time to the concentration of control tissue. In view of the possibility that isocitric dehydrogenase and oxalosuccinic carboxylase activities are due to the same catalytic protein, the carboxylase activity was also measured in control and copper-deficient extracts. There was a proportional increase of both activities in the copper-deficient material. The above changes in enzyme constitution are attributed to a role of copper in protein metabolism.

Chemical abnormalities in the blood of the immature infant. SAMUEL NATELSON. *Dept. of Biochemistry, Rockford Memorial Hospital, Rockford, Ill.*

This is a preliminary report on a study initiated in cooperation with the Department of Health of the City of Rockford to see whether chemical evidence could be found for the cause of the high fatality rate in premature infants. Analytical methods were developed for following daily the electrolyte, protein, hematocrit, pH and CO_2 levels using capillary blood. Included in this study were full-term infants who from birth showed pathological symptoms, from no obviously apparent anatomical cause. The most consistent finding in these infants was an inability to retain sodium chloride and water with a tendency to retain potassium. Thus in contrast to the normal infant, the hematocrit value of their blood tended to remain elevated, their sodium and chloride level tended to remain low and potassium level high. These symptoms are commonly associated

with adrenal insufficiency. Cortisone was apparently ineffective in causing retention of sodium and chloride in these cases but desoxycorticosterone acetate was very effective. Sodium chloride and desoxycorticosterone administered daily in amounts designed to maintain electrolyte blood levels at essentially normal values was very effective in maintaining these infants in normal health. The hematopoietic system seems to be impaired in these infants and under treatment (several months) a steady drop in hematocrit value is observed requiring repeated transfusions. There is apparently no relationship between the incidence of adrenal immaturity and birth weight. In the 10 cases of adrenal immaturity encountered, the birth weights ranged from 800 gm. to 5 kg. Several prematures weighing in the order of 1 kg. did not show this abnormality.

Reaction of the trypsin system with diisopropylfluorophosphate (DFP). HANS NEURATH, LEON W. CUNNINGHAM, JR.,* FRANK TIETZE* AND N. MICHAEL GREEN.* *Dept. of Biochemistry, Univ. of Washington, Seattle.*

DFP reacts with trypsin (JANSEN *et al.*, *J. Biol. Chem.* 179: 189, 1948) but not with trypsinogen or with crystalline trypsin-pancreatic inhibitor compound. Crystalline DFP-trypsin has been isolated from solutions which contain a DFP/trypsin mole ratio not exceeding 3:1 at pH 8. DFP-trypsin has the same crystal form and N content (15.0%) as active trypsin. The P content of various crystalline preparations was variable, i.e. 0.14% and 0.22%, respectively. Recrystallized preparations contain less than 0.2% tryptic activity. DFP-trypsin is unreactive toward pancreatic or soybean inhibitor. The sedimentation behavior of DFP-trypsin is characteristic of that of a single molecular species over the pH range of 3.86–7.8. Linear extrapolation to zero protein concentration yields a value of $s_{20,w}$ of 2.5 Svedberg units which is also approached by active trypsin. However, at finite protein concentrations, the sedimentation behavior of active trypsin is characteristic of reversible, concentration dependent molecular aggregation, maximum aggregation occurring at pH 5.

Isolation of progesterone from human placentae. MATTHEW W. NOALL, HILTON A. SALHANICK AND M. X. ZARROW (introduced by MARVIN D. ARMSTRONG). *Dept. of Biological Chemistry, Univ. of Utah School of Medicine, Salt Lake City, and Dept. of Biological Sciences, Purdue Univ., Lafayette, Ind.*

Progestational activity has been reported in human blood and placentae but the presence of progesterone could not be demonstrated. This has led some workers to conclude that a substance other than progesterone is the active factor. We are reporting the isolation of approximately 10 mg. of progesterone from 11.2 kg. of normal postpartum human placentae. Finely ground placentae were treated for 2 days at room temperature with an equal volume of 5% NaOH and then extracted with ether. Ketones were separated with Girard's Reagent T and initial purification was achieved by fractional chromatography. Fractions containing the progestational activity were acetylated, and subjected to further chromatography, followed by sublimination of the purified fractions. A white crystalline solid was obtained, m.p. 124–125°; mixed m.p. with progesterone, 123.5–125° (progesterone, m.p. 123–125°). The isolated substance had an absorption maximum at 240 mμ. The absorption spectra of the dinitrophenylhydrazine derivative and known progesterone *bis*dinitrophenylhydrazone were the same and their melting points were 288–294° and 287–297° respectively. The Hooker-Forbes microbioassay procedure was used as a guide in the chemical isolation. The amount of isolated progesterone was sufficient to account for the known progestational activity of the starting material.

Biochemical heterogeneity of the cytoplasmic particles isolated from rat liver homogenate. ALEX B. NOVIKOFF, ESTELLE PODBER, JEAN RYAN AND ELSIE NOE (introduced by HAROLD B. PIERCE). *Depts. of Pathology and Biochemistry, Univ. of Vermont College of Medicine, Burlington.*

The question of biochemical heterogeneity among the cytoplasmic granules has been investigated by separating 0.88 M sucrose homogenates of rat liver, by differential centrifugation, into 10 fractions: 'nuclear fraction,' 8 granule fractions and 'supernatant fluid.' Of the 8 granule fractions, 3 were essentially mitochondrial, 2 were mixtures of mitochondria and microsomes, and 3 were essentially microsomal. Marked chemical and enzymatic heterogeneity was found among the isolated microsome fractions and relatively minor heterogeneity among the mitochondrial fractions. The more readily sedimented (larger?) microsomes are rich in PNA and show high esterase, acid phosphatase and uricase activities, while the less readily sedimented (smaller?) microsomes, although rich in PNA and esterase, have low acid phosphatase and uricase activities. Other less striking differences among the microsomes were observed in the levels of 5′-nucleotidase and alkaline phosphatase. The 3 mitochondrial fractions revealed minor differences in their levels of acid phosphatase, uricase, ATP-ase, succinoxidase and alkaline phosphatase. This work has led to a schedule of centrifugation by means of which one may isolate

the following fractions: *1*) relatively uncontaminated mitochondria, *2*) a mixture of small mitochondria and microsomes, *3*) a mixture of 'large' and 'small' microsomes, and *4*) 'small' microsomes. It is suggested that the unnecessarily high centrifugal forces frequently used to sediment mitochondria and differences in the technique of handling the 'poorly-sedimented pink layer' account for the wide variations in values reported for the PNA content, uricase activity and acid phosphatase activity of isolated mitochondria.

Kinetics of acid hydrolysis of pentose nucleic acid derivatives. ODDVAR NYGAARD* AND CYRUS P. BARNUM. *Dept. of Physiological Chemistry, Univ. of Minnesota Med. School, Minneapolis.*

The hydrolysis of pentose nucleic acid derivatives by strong acid at 100°C has been studied. The first order rate constants for the hydrolysis of the N-glycosidic bond in the purine derivatives guanosine, adenosine, adenosine-5′-phosphoric acid and adenosine-3′-phosphoric acid in N H_2SO_4 have been determined, their values being 0.54, 0.66, 0.177 and 0.103 min.$^{-1}$, respectively. The liberation of phosphoric acid from the 2 adenine nucleotides was also measured and tentative rate constants for the hydrolysis of the derived ribose phosphates have been calculated, being 0.071 min.$^{-1}$ for ribose-3-phosphate and 0.0018 min.$^{-1}$ for ribose-5-phosphate. Under the same conditions the rate constants for the direct liberation of phosphoric acid from uridylic and cytidylic acids are 0.00044 and 0.00046 min.$^{-1}$. Approximate rate constants for the liberation of nitrogen base from pyrimidine nucleosides and nucleotides in 8.6 N HCl have also been determined, having the values 0.015, 0.0061, 0.0029 and 0.00052 min.$^{-1}$ for uridine, uridylic acid, cytidine and cytidylic acid, respectively.

***Clostridium histolyticum* proteinase.** JAMES D. OGLE (introduced by ALFRED A. TYTELL). *Dept. of Biological Chemistry, Univ. of Cincinnati, Cincinnati, Ohio.*

Clostridium histolyticum proteinase was prepared essentially free of collagenase activity. When activated by cysteine the proteinase hydrolyzed the following synthetic substrates: L-arginine methyl ester, α-N-benzoyl-L-arginineamide and α-N-benzoyl-L-arginine ethyl ester. The hydrolysis of esters of benzoylarginine was qualitatively independent of the ester groups, since the ethyl ester, the benzyl ester, and the isopropyl ester were all hydrolyzed by the activated proteinase. The rates of hydrolysis of α-N-benzoyl-L-arginineamide and α-N-benzoyl-L-arginine ethyl ester were identical. L-Lysine methyl ester was hydrolyzed very slowly. Chymotrypsin substrates, such as L-leucine methyl ester, DL-Phenylalanine methyl ester, DL-phenylalamineamide, were not hydrolyzed. A papain substrate, N-benzoylglycineamide, was not hydrolyzed. Peptidase activity was shown to be absent by the lack of hydrolysis of L-leucylglycylglycine, glycylglycylglycine, glycylglycine, and L-leucineamide. The pH optimum for the hydrolysis of synthetic substrates by the activated proteinase was about 7.2. The proteinase was activated by cysteine, glutathione and to a lesser extent by cyanide. The activated proteinase was markedly inhibited by borate, citrate and veronal. The proteinase can be highly purified by fractionation with very low alcohol concentrations in the presence of zinc salts. The use of synthetic substrates for *Cl. histolyticum* proteinase affords a consistent and reproducible method for characterization and assay of this enzyme.

Nucleotide content of cellular components from human fetal liver. PETER S. OLMSTED (introduced by CLAUDE A. VILLEE). *Dept. of Biological Chemistry, Harvard Med. School, Boston, Mass.*

Human fetal liver, obtained at therapeutic interruptions of pregnancy, was homogenized in a Potter glass homogenizer, suspended in 0.25 M sucrose and separated into nuclear, mitochondrial, microsomal and supernatant fractions by differential ultracentrifugation (HOGEBOOM AND SCHNEIDER. *J. Biol. Chem.* 186: 417, 1950). Each cellular component was then fractionated by a modified Ogur-Rosen procedure (*Arch. Biochem.* 25: 262, 1950) in which, after lipids and acid-soluble compounds had been removed, the ribonucleic acid was extracted with 1.6 N cold perchloric acid and the desoxyribonucleic acid was subsequently extracted with hot perchloric acid. To insure complete hydrolysis the nucleic acid from each fraction was then hydrolyzed with 1 N KOH. The nucleotides of each fraction were separated by ion exchange chromatography (COHN. *J. Am. Chem. Soc.* 72: 1471, 1950) and their amounts determined spectrophotometrically. Estimates of the nucleic acid content of each fraction, made from pentose, phosphorus and ultra-violet absorption data, agreed within 15%. Essentially quantitative recoveries of the nucleotides from each cellular fraction were achieved and both molar ratios and mg. nucleotide/gm. tissue in each of the cellular fractions will be presented. Both nuclear and mitochondrial ribonucleotides have similar molar ratios and are rich in guanylic and cytidylic acids.

Physical characterization and kinetic properties of beef liver glutamic acid dehydrogenase. JAMES ALLEN OLSON (introduced by CHRISTIAN B. ANFINSEN). *Dept. of Biochemistry, Harvard*

Med. School, Boston, Mass., and Natl. Heart Inst., Natl. Insts. of Health, Bethesda, Md.

Physical properties of glutamic acid dehydrogenase, which appeared to be monodisperse in Na_2SO_4 fractionation, recrystallization, ethanol fractionation, and electrophoretic studies were: partial specific volume, 0.75 cc/g.; $S_{20.w}$, 26.6 × 10^{-13} sec.; $D_{20.w}$, 2.54 × 10^{-7} cm²/sec.; MW, 1,000,000 g/mole; f/f$_o$, 1.26. $E^{1\%}_{1cm}$ at 279mμ was 9.7, and $E_{279m\mu}/E_{260m\mu}$ was 1.6. The progressive decrease in the sedimentation constant at enzyme concentrations below 0.27% suggested that dissociation or unfolding occurred at high dilutions. The turnover number (moles DPN/min/10^5 gm. enzyme) was 290 for DPN reduction and 3000 for DPNH oxidation. The pH optima for various systems in different buffers were: glutamate-DPN, 8.0 (histidine), 7.7 (phosphate); glutamate-TPN, 8.0 (histidine), 7.7 (veronal); glutamate-desamino DPN, 8.1–8.5 (histidine), and alpha ketoglutarate-NH_4-DPNH, 7.6 (veronal). At the optimal pH the relative velocities with DPN, desamino DPN and TPN were 100: 60:35, respectively. Of 14 compounds tested, only l-glutamate was oxidized. The Michaelis constants at 25°, pH 7.6 were: DPN, 10.1 × 10^{-5}M; DPNH, 9.6 × 10^{-5}M; TPN, 5.7 × 10^{-5}M; desamino DPN, 9.1 × 10^{-5}M; l-glutamate, 1.1 × 10^{-3}M; alpha ketoglutarate, 0.7 × 10^{-3}M; NH_4 = .056M. Equilibrium constants determined at 27° at various reactant concentrations were: DPN, 6.89 × 10^{12} L2/M², −17.6 kg. cal/mole; TPN, 10.13 × 10^{12} L2/M², −17.9 kg. cal/mole; and desamino DPN, 10.3 × 10^{12} L2/M², −17.9 kg. cal/mole. Equilibrium data indicated the absence of NH_4-stabilized alpha iminoglutarate. Assuming an E^1_0 for DPN/DPNH of −0.282v. (Borsook, H., *J. Biol. Chem.* 133: 629, 1940), derived E^1_0 values were: TPN/TPNH, −0.287v.; desamino DPN/desamino DPNH, −0.287v., and alpha ketoglutarate × NH_4/glutamate, −0.107v. Inhibitor concentrations causing 50% inhibition of the enzyme (3.3 μ/cc.) at pH 7.6, 26° were: *p*-chloro-mercuribenzoate, 1.1 × 10^{-4}M; $AgNO_3$, 3.2 × 10^{-7}M; $HgAc_2$, 3.5 × 10^{-6}M; $ZnSO_4$, 1.7 × 10^{-4}M; $FeCl_3$, 4.4 × 10^{-4}M. The activation energy was 14,000 calories/mole.

Rate of appearance of bound-I^{131} in the blood. Charles A. Owen, Jr.* and Marschelle H. Power. *Divs. of Clin. Pathology and Biochemistry, Mayo Clinic, Rochester, Minn.*

A series of patients with hyperthyroidism were studied while they were receiving therapeutic amounts of radioactive iodine. Thyroid uptake of orally administered NaI^{131} (allocated by the A.E.C.) was determined directly by *in vivo* measurement, and by calculation from urinary excretion data. Bound I^{131} was calculated by an adaptation of the principle of Scott, Reavis, Saunders and White (*Proc. Soc. Exper. Biol. & Med.* 76: 592, 1951): the erythrocyte/plasma ratio of NaI^{131} was found to be 0.56; any reduction in this value indicated the presence of erythrocyte-impermeable I^{131} in the plasma, such as I^{131}-albumin added *in vitro* or of 'protein-bound I^{131}' in the case of treated patients. From the observed ratio, absolute values for permeable and impermeable I^{131} were calculated. By serial determinations in hyperthyroid persons the impermeable I^{131} was found to appear in the plasma within a few hours of the administration of I^{131}, and to reach a peak in 3 or more days. There was a limited correlation between the uptake and release of I^{131} by the thyroid.

Chemical properties of protogen. Ernest L. Patterson and John A. Brockman, Jr. (introduced by T. H. Jukes). *Lederle Labs. Div., American Cyanamid Co., Pearl River, N. Y.*

Protogen A, the predominant form in alkaline hydrolyzed insoluble liver fraction, is converted to Protogen B by mild oxidizing agents. Elementary analysis of Protogen B indicates the presence of C, H, S. By titration with sodium hydroxide an acid equivalent of 230 and a saponification equivalent of 127 were found. Protogen A gave a negative nitro prusside test for thiol which became positive after treatment with cyanide. Protogen B gave a negative nitro prusside test for thiol. However, after saponification 1 equivalent of thiol was titrated with iodine. Following reduction of Protogen B with sodium borohydride 2 equivalents of thiol were titrated with iodine. The reduced Protogen B was easily oxidized to a compound having properties identical with those of Protogen A.

Transfructosidase, a fructosyl-transferring enzyme of *Aspergillus oryzae*. John H. Pazur (introduced by Otto A. Bessey). *Dept. of Biological Chemistry, Univ. of Illinois College of Medicine, Chicago, Ill.*

A new carbohydrate-transferring enzyme, transfructosidase, has been isolated from cultures of the mold *Aspergillus oryzae*. The enzyme was separated from other carbohydrases in the mold filtrate by chromatography on starch columns. Transfructosidase effects transfructosidation reactions in which fructosyl units of sucrose or raffinose are transferred to co-substrate saccharides either sucrose, fructosyl-sucrose or raffinose. From sucrose the enzyme synthesized 2 new oligosaccharides, a trisaccharide of 1 glucose and 2 fructose residues, and a tetrasaccharide of 1 glucose and 3 fructose residues. The enzyme disproportionated raffinose to yield a new tetrasaccharide, fructosyl-raffinose, and a disaccharide, melibiose, but did not act on melezitose or tura-

nose. A reaction mixture of raffinose, C^{14}-sucrose (labeled in the glucose and the fructose moieties) and transfructosidase contained the radioactive disproportionation products of sucrose and a radioactive tetrasaccharide of the raffinose series. The latter was synthesized by the transfer of C^{14}-fructosyl units of radioactive sucrose to nonradioactive raffinose. The tetrasaccharide was isolated by paper chromatography and degraded enzymatically into fructose and melibiose. Examination of the degradation products for radioactivity showed that only the fructose was radioactive.

Estrogen metabolism in human pregnancy: a study with the aid of deuterium. W. H. PEARLMAN, M. R. J. PEARLMAN* AND A. E. RAKOFF.* *Depts. of Biochemistry and of Obstetrics and Gynecology, Jefferson Med. College, Philadelphia, Pa.*

The 6,7-d_2-estrone acetate was prepared in this laboratory (*J. Am. Chem. Soc.* 72: 5781, 1950) and administered intramuscularly to women in late pregnancy. The urine was subsequently collected, hydrolyzed with acid, and extracted; the phenolic material was partitioned into 'estrone,' 'estradiol,' and 'estriol' fractions; bioassays for estrogenic activity were performed. Estriol was isolated in crystalline form and was found to contain significant amounts of excess deuterium. The urinary estrone had a relatively high content of deuterium; the determination in this case was performed by application of an isotope dilution technique; Preliminary results indicate that there is an *in vivo* conversion of estrone to estriol during pregnancy but the recovery of the injected isotopic estrogen in the form of urinary isotopic estriol is low. The rate of estrogen destruction and elaboration in late pregnancy and the influence of progesterone will be discussed.

Hippuric acid synthesis, plasma glycine level and tissue glutathione concentration in healthy persons under psychological stress. HAROLD PERSKY (introduced by THORFIN R. HOGNESS). *Inst. for Psychosomatic and Psychiatric Research and Training, Michael Reese Hosp., Chicago, Ill.*

Mentally and physically healthy persons when exposed to psychological stresses ranging from mild (college examinations) to severe (parachute-jump training), excrete an elevated amount of hippuric acid following sodium benzoate administration. This elevated amount of hippuric acid is not as great as that produced by subjects with anxiety states (PERSKY, GRINKER AND MIRSKY. *J. Clin. Investigation* 29: 110, 1950). The increased hippuric acid production is not due to an elevated plasma glycine or amino acid level. Red blood cell glutathione level is significantly lowered in these subjects. Work is in progress to determine whether this substance is responsible for the increased hippuric acid production in individuals under stress.

Crystalline pyridoxamine and pyridoxal phosphates. ELBERT A. PETERSON,* HERBERT A. SOBER AND ALTON MEISTER. *Natl. Cancer Inst., Natl. Insts. of Health, Bethesda, Md.*

Pyridoxal phosphate is believed to be the coenzyme for transaminases, amino acid decarboxylases, and other systems. Pyridoxamine phosphate, which has been reported to occur in nature, may be involved in the catalysis of transamination, but this has not yet been established. Since only impure preparations of these phosphate esters have been available, it appeared desirable to prepare the pure compounds for use in enzymatic and other investigations. Crystalline pyridoxamine phosphate has now been prepared by reaction of pure pyridoxamine with anhydrous phosphoric acid followed by chromatographic purification on a weak cation exchange resin. Crystalline pyridoxal phosphate was obtained as the sodium salt by oxidation of pure pyridoxamine phosphate and ion exchange chromatography. The details of the preparative procedures and the chemical and enzymatic characterization of these compounds will be presented. Pyridoxamine phosphate or pyridoxal phosphate activated a resolved purified glutamic-aspartic pig heart transaminase system to about the same extent. Activation by both phosphate esters was prevented by simultaneous addition of a product obtained by phosphorylation of desoxypyridoxine. Considerably less inhibition was observed when the relative concentration of pyridoxal phosphate was increased, or when pyridoxal phosphate was incubated with the enzyme prior to addition of inhibitor. The evidence suggests that the inhibitor interferes with coenzyme-apotransaminase combination. The implications of these findings will be discussed.

Insulin fibril formation: application to the isolation of S-35 labeled insulin. C. W. PETTINGA AND C. N. RICE (introduced by O. K. BEHRENS). *Lilly Research Labs., Indianapolis, Ind.*

The conventional methods of insulin isolation are not satisfactory when applied to small amounts, 10–100 gm., of pancreas tissue. Although insulin fibril formation has been used successfully for insulin assay, it has not been applied extensively for the isolation of physiologically active insulin. We have used it for the isolation of insulin-S-35. Surviving beef pancreas slices were incubated with S-35 labeled methionine in bicarbonate buffered Ringer's solution. The slices were homogenized in the cold incubation solution with a Waring blendor and the resulting slurry was lyophilized. After extraction of the dried residue with 60% ethanol at pH 1.9, the insulin

was precipitated, along with impurities, by the addition of 4 volumes of ether and 2 volumes of absolute ethanol. This precipitate was extracted with 0.05 N hydrochloric acid solution. After seeding with carefully prepared insulin fibrils, the acid extract was incubated at 47°C. for 6 hours. The resulting precipitate of insulin fibrils was removed by centrifuging, and was washed with dilute sodium sulfate solution at pH 1.6. The washed fibrils were dissolved in cold 0.05 N sodium hydroxide to obtain a preparation with full physiological activity after appropriate dilution with isotonic buffer solution. These preparations were, however, not stable at room temperature. Significant decomposition occurs in 3 hours. A typical 5-mg. insulin preparation contained 1600 c/min/mg. when 0.15 mc. of DL-methionine was used with 50 gm. of pancreas slices. Methods used for assay of physiological activity and for determining purity of the preparation will be presented.

Further characterization of pseudovitamin B_{12} pigments. J. J. PFIFFNER, H. W. DION* AND D. G. CALKINS.* *Research Labs., Parke, Davis and Co., Detroit, Mich.*

The harvest broth of a rumen anaerobe has yielded 3 pairs of pigments of the pseudo (ψ) series; 3 cyano-cobalt pigments and the corresponding 3 presumably hydroxo compounds. Two pairs (crystalline) are highly active as growth factors for certain lactobacilli but are inactive in chicks, rats and man (pernicious anemia). The 3rd pair (*ca.* 30% pure) have no demonstrable biological activity but exhibit ultraviolet absorption properties typical of the series. Two crystalline cyano pigments (ψ-vitamins B_{12} and B_{12b}) when subjected to acid degradation have yielded Dg-1-aminopropanol-2, inorganic phosphate, ammonia, a red cobalt fraction, adenine and a trace of hypoxanthine (paper chromatographic evidence). The hypoxanthine is considered to arise from the deamination of adenine. No indication was obtained for the occurrence of 5,6-diemthylbenzimidazole or its derivatives. The crystalline pigments of the ψ-series apparently contain adenylic acid in place of 5,6-diemthylbenzimidazole-1-α-D-ribofuranosido-phosphate. The presence of adenine instead of 5,6-dimethylbenzimidazole is in keeping with the higher nitrogen and lower carbon content of the ψ-vitamins as compared to vitamin B_{12} itself, (ψ-vitamin B_{12}: C, 52.06; H, 6.60; N, 17.85; Co, 4.40; P, 2.31. ψ-vitamin B_{12b}: C, 52.45; H, 6.51; N, 18.62; Co 4.34; P, 2.28.) Following the cobalamin system of nomenclature for vitamin B_{12}, ψ-vitamin B_{12} may be referred to as cyano-β-cobalamin and ψ-vitamin B_{12b} as cyano-γ-cobalamin.

Mean serum calcium and phosphorus levels in infants fed formulas containing and not containing vitamin D during the first week of life. JOSEPH B. PINCUS AND ISAAC F. GITTLEMAN (introduced by BENJAMIN KRAMER). *Pediatric Research Labs., Jewish Hosp. of Brooklyn, Brooklyn, N. Y.*

It is an established fact that the placental barrier does not permit the passage of fat-soluble vitamins from the mother to the fetus. Newborn infants are therefore given supplements of these vitamins, especially vitamin A and D, almost from birth. Though the amount given varies, it seldom exceeds more than 100 U.S.P. U of vitamin D/day during the first week of life. Park, Guy and Powers and, independently, Kramer and Howland have demonstrated that vitamin D has a regulatory effect upon the serum calcium and phosphorus levels in rats fed diets containing varying amounts of calcium and phosphorus. In an attempt to explain the apparent increase in the incidence of neonatal tetany we decided to study the effect of vitamin D on the serum calcium and phosphorus levels in infants during the first week of life. Seven groups of infants were thus studied: *Group 1* consisted of 41 infants fed evaporated milk mixtures containing 25 U of vitamin D/ounce of undiluted milk. *Group 2* consisted of 17 infants fed fresh cow's milk fortified with 400 U of vitamin D/quart. *Group 3* numbered 18 infants who received cow's milk not fortified with vitamin D. *Group 4* comprised 60 infants who received powdered milk fortified with vitamin D. *Group 5* consisted of 60 infants who were fed powdered milk not fortified with vitamin D. *Group 6* comprised 18 infants who were fed breast milk supplemented with powdered milk not containing vitamin D. *Group 7* consisted of 17 infants who were breast fed only. The nursing mothers did not receive supplements of vitamin D in their diets. Serum calcium and phosphorus were determined on the first day and the day of the discharge. It is evident that vitamin D, in the amounts given, had no regulatory effect on the serum calcium and phosphorus levels; in fact the tendency to hypocalcemia was more marked in the groups of infants who received formulas containing vitamin D. Twenty-four and four tenths % of infants fed evaporated milk and 17.6% of the infants who were fed cow's milk fortified with vitamin D developed hypocalcemia while no instance of hypocalcemia was observed in the infants who were fed cow's milk not containing vitamin D. In the groups of infants fed powdered milk the incidence of hypocalcemia is twice as high in the infants fed the vitamin D containing milk. In the breast-fed groups there was no incidence of hypocalcemia.

Oxidative metabolism of heart particulate preparations. G. W. E. PLAUT AND KATHERINE L. PLAUT (introduced by W. H. PETERSON).

Inst. for Enzyme Research and Dept. of Biochemistry, Univ. of Wisconsin, Madison.

Guinea pig heart ventricles were homogenized in 0.25M sucrose, centrifuged (600 *g*), and the supernatant centrifuged at 5000 *g*. The particles thus obtained were washed and used for the respiration studies. Succinate, malate, and α-ketoglutarate were completely oxidized by these preparations (Q_{O_2}(N) 200–600). Pyruvate and acetate required a trace of malatefor optimum oxidation (Q_{O_2}(N) 200–300). Citrate and cisaconitate were not oxidized although whole homogenates did metabolize them. Though citrate was inert the following observations support the view that acetate was oxidized via the tricarboxylic acid cycle: *1*) in the presence of malate there was net formation of citrate from acetate—this was enhanced by fluoroacetate: *2*) $C^{14}H_3COOH$ oxidized in the presence of a pool of α-ketoglutarate and 0.01M malonate yielded radioactive α-ketoglutarate and succinate, and nonradioactive respiratory CO_2; *3*) $C^{14}H_3COOH$ was oxidized with a citrate pool. Reisolated citrate contained radioactivity, and respiratory CO_2 indicated dilution. However, the specific activities of the compounds indicated that formed and added citrate were *not* in complete equilibrium. Investigation of the cause of lack of citrate oxidation revealed that washed particles contained only 5% of the isocitric dehydrogenase of the homogenate. Most of this activity was in the supernatant. Oxidation of citrate by washed particles was restored by certain TPN and DPN preparations, but highly purified TPN, DPN, and 5-adenylate were inactive. However, as little as .005 μM/ml. of nicotinamide mononucleotide activated citrate oxidation.

Oxidative phosphorylation in spleen mitochondria. Richard L. Potter and Frank H. Bethell (introduced by M. E. Swendseid). *Atomic Energy Commission, Biological effects of Irradiation Lab., Univ. of Michigan, Ann Arbor.*

Homogenized rat spleen fractionted in isotonic sucrose by the method of Schneider (*J. Biol. Chem.* 176: 259, 1948) yields mitochondria which have the capacity to carry out oxidative phosphorylation. Citrate, glutamate and α-ketoglutarate are oxidized slowly for about 20 minutes. Pyruvate is oxidized at a rapid but progressively diminishing rate for 15 minutes. Under the conditions of the succinoxidase assay succinate is oxidized at a steady rate for 2 hours. In a medium containing AMP, magnesium, cytochrome *c*, phosphate, glucose, hexokinase and 0.3 mg. of mitochondrial nitrogen/3 ml. only succinate will cause consistent phosphate esterification. Under these conditions 100–200γ of P can be esterified in 20 minutes at 38°C. with a maximum P/O ratio of 0.8. The mitochondria from rats given a lethal dose of x-irradiation (800γ) show a reduced capacity to carry out oxidative phosphorylation. In 11 experiments the mitochondria from control animals esterified more P in every case (average difference = 106γ). This difference can be detected as early as 1 hour after irradiation and after 24 hours the P/O ratio is usually negative. Normal mitochondria given 2000 r of x-irradiation *in vitro* at 2°C. in isotonic sucrose are unaffected. This may mean that the reduced phosphorylation of mitochondria irradiated *in vivo* is due to a physiological response of the animal to irradiation rather than a direct effect of irradiation on the mitochondria.

Latent ATP-ase in rat liver mitochondria. Van R. Potter and H. C. Simonson.* *McArdle Memorial Lab., Univ. of Wisconsin, Madison.*

Mitochondria from 25 mg. of rat liver were tested for ATP-ase activity in a volume of 2 ml. at 30° in isotonic sucrose containing 4 μM of ATP. In the control tubes, less than 0.5 μM of inorganic phosphate (IP) was liberated in 60 minutes. With magnesium added (.002 M final) there was no IP formation for 30–40 minutes, after which time a rapid release of IP occurred. With magnesium plus calcium (.001 M) the IP formation was maximal at once and reached a plateau at approximately 8 μM after 60–90 minutes. With dinitrophenol (3×10^{-5} M) alone or with calcium alone, IP formation began at once but occurred at less than the maximal rate. When mitochondria were *preincubated* aerobically at 30° for 3 hours, or subjected to a variety of other treatments, and then tested as above, they responded maximally to Mg *alone* but were inactive in its absence even when DNP or calcium were added. Assays for ATP-ase are undoubtedly complicated by these effects. Evidently Ca and DNP are unable to activate ATP breakdown in mitochondria directly but act via a mechanism present in fresh mitochondria and lacking in preincubated mitochondria. The operation of this mechanism probably is sufficient to account for most of the known effects of DNP.

Serum lipotroteins in human atherosclerosis. Hugh M. Pratt (introduced by Elmer H. Stotz). *Depts. of Medicine and Biochemistry, Univ. of Rochester, Rochester, N. Y.*

Alpha lipoproteins and beta lipoproteins have been prepared from the serum of individual persons by the method of high speed centrifugation. The sera were given an increment in specific gravity through the addition of NaCl or KBr prior to centrifugation in the preparatory ultracentrifuge. High speed centrifugation for varying periods of time results in layering of the lipoproteins at the top of the tube. The lipoproteins were

pipetted off as quantitatively as possible. Aliquots were analyzed for protein by the biuret method, for cholesterol by the method of Schoenheimer and Sperry, and for lipid phosphorous by the method of Kuttner and Lichtenstein. It was possible to isolate beta lipoproteins and/or a mixture of alpha and beta lipoproteins largely free from other serum proteins by this method. Occasional runs in the analytical ultracentrifuge were used to check the purity of preparations. Preliminary results in a group of persons without known cardiovascular disease and a group of persons with recent myocardial infarction have been obtained. These indicate that an increase in beta lipoprotein and a decrease in alpha lipoprotein characterize the latter group. The proportions of cholesterol, phospholipid, and protein are markedly different in the alpha and beta lipoprotein fractions, but no variations in composition within either fraction have thus far been noted.

Occurrence of an acetone-soluble uncoupling agent in rat liver microsomes. BERTON C. PRESSMAN (introduced by M. A. STAHMANN). *Dept. of Biochemistry and Inst. for Enzyme Research, Univ. of Wisconsin, Madison.*

While investigating the effects of alkali cations on rat liver mitochondria, it was found that addition of the microsomal fraction makes apparent a K^+ requirement for optimal enzymatic activity. This property of intact microsomes is retained after boiling and is exhibited by acetone extracts but not by the acetone extracted residue. Both intact microsomes and the acetone extract stimulate mitochondrial respiration with Krebs cycle intermediates, depress the P/O ratio, and, in the absence of oxidizable substrate, cause mitochondria to break down ATP. This latter property was developed into an assay for the active agent of microsomes. The active agent is not completely destroyed by refluxing with alcoholic KOH and contains neither free acidic nor basic groups. Its biological properties were compared extensively with those of dinitrophenol and desoxycholic acid, which are capable of producing similar effects on mitochondria, and found to be significantly different. The microsomal agent is able to stimulate mitochondrial respiration as well as dinitrophenol does, yet the maximum rate of ATP breakdown induced by the microsomal agent is only one half that achieved with dinitrophenol. Dinitrophenol added to mitochondria in the presence of the microsomal agent fails to elicit any effect beyond that produced by the microsomal agent alone. A preparation possessing similar properties has been obtained from pig hearts.

6-Azathymine as an antimetabolite. WILLIAM H. PRUSOFF AND WILLIAM L. HOLMES (introduced by A. D. WELCH). *Dept. of Pharmacology, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

3,5-Dihydroxy-6-methyl-1,2,4-triazine (6-azathymine) has been synthesized by the method of Bailey and Knox (*J. Am. Chem. Soc.* 29: 886, 1907) and some of its biologic activities have been investigated. Although azathymine will not replace the thymine requirement of *Streptococcus faecalis* (A.T.C.C. 8043) or of *Lactobacillus casei* (A.T.C.C. 7469) in a medium deficient in pteroylglutamic acid, it functions as a competitive antagonist of thymine. For essentially complete inhibition of bacterial growth molar ratios of antimetabolite to metabolite of approximately 45 (*S. faecalis*) and 1500 (*L. casei*) are required. Studies of the effect of azathymine on these organisms in the presence of thymidine and pteroylglutamic acid are in progress. Azathymine has shown toxic effects of significance on the development of chick embryos and this phenomenon is being investigated further.

Origin and nature of abnormal proteins in multiple myeloma. FRANK W. PUTNAM. *Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.*

In continuing study of the origin and nature of abnormal serum proteins in multiple myeloma and their relationship to Bence-Jones proteins (BJ), (*Federation Proc.* 10: 262, 1951), emphasis has been laid on the protein increment which migrates electrophoretically as 'beta' globulin. In sera screened electrophoretically and selected for this abnormality the beta component comprised up to 70% of the total proteins. Ultracentrifugal analysis of whole sera revealed a component sedimenting more rapidly than normal globulin. Electrophoretically isolated abnormal protein contained two sedimenting components, ($S_{20} = 6.5$ and $S_{20} = 8.5$), but the distribution was not always correlated with electrophoretic analysis. BJ proteins excreted in several cases have been characterized by physical methods. Since no evidence on the relationship of abnormal serum and urinary proteins was obtained from physical data, isotopic study was begun. A patient excreting a homogenous BJ protein ($S_{20} = 3$) and with serum containing 60% of a beta type protein ($S_{20} = 8$, major component, $S_{20} = 6$, minor component) was given $CH_2(NH_2)\ C^{13}OOH$. BJ protein was isolated by precipitation at 3 M ammonium sulfate. Albumin and abnormal globulin were separated by salt precipitation and by electrophoresis. In work now in progress the glycine of each protein will be isolated chromatographically and the isotopic activities compared. The diversity in serum protein response to this disease, manifested in electrophoretic patterns, suggests the need for further isotopic study and comparison of selected cases with established data for normal protein turnover.

Enzyme inhibition by snake venoms in relation to their neurotoxic action. J. H. QUASTEL AND B. M. BRAGANCA.* *Research Inst., Montreal General Hosp., Montreal, Canada.*

Snake venoms heated at 100°C. for 15 minutes retain their toxicity to the animal. The effects of such heated venoms on 24 enzyme systems have been investigated. The enzymic systems concerned with the oxidation of glucose, pyruvate, glutamate, succinate, α-ketoglutarate and fructose by brain homogenates are inactivated by small quantities of the heated venoms. Pyruvic dehydrogenase of brain, succinic dehydrogenase of brain and heart, cytochrome oxidase of brain and choline oxidase of liver are all vigorously attacked. The following systems are not attacked: anaerobic glycolysis by rat brain, glucose fermentation by yeast extract, pyruvic acid dismutation by pigeon liver, hexokinase, glucose oxidase of liver and glucose oxidase of fungi (notatin), soluble pyruvic dehydrogenase of pigeon breast muscle, lactic and malic dehydrogenases and urease. Cytochrome *c* is not broken down. The enzyme systems that are not attacked by heated venom are distinguished from those that are attacked by being active in aqueous cell-free tissue extracts and are apparently independent of cellular structures for their activities. Heated snake venom contains a heat stable lecithinase that attacks brain phospholipoids or lipoproteins. Its inhibiting effect on a susceptible enzyme system is enhanced by calcium ions that activate lecithinase. It is concluded that heated venoms exert their effects by enzymatic degradation of mitochondrial structures upon whose integrity or particular spatial configurations the enzyme systems concerned depend for their optimal activities.

Bile and the absorption of vitamin K_1. ARMAND J. QUICK AND CLARA V. HUSSEY.* *Dept. of Biochemistry, Marquette Univ. School of Medicine, Milwaukee, Wis.*

After cholecystnephrostomy a dog gradually developed a severe hypoprothrombinemia. The daily intravenous administration of 0.5γ of vitamin K_1/kg. of body weight maintained the prothrombin at the normal level. On withdrawing the maintenance dose, the prothrombin consistently dropped to 5% of normal or lower in one week. Feeding a gram of desiccated bile daily for a week had no counteracting effect on the hypoprothrombinemia. Even when 2 mg. of vitamin K_1 were given with the bile, no increase in prothrombin occurred. On increasing the amount of vitamin K_1 to 5 mg. a distinct augmentation of prothrombin was observed, but even 100 mg. of emulsified vitamin K_1 (Merck) with bile only restored the prothrombin to 50% of normal. On the basis of the amount of vitamin K_1 that when given intravenously corrected completely the hypoprothrombinemia, it appeared that only about 20γ of vitamin K_1 were absorbed, which is 0.02% of the amount fed. Water-soluble synthetic vitamin K compounds promptly corrected the hypoprothrombinemia when given orally. Thus a single dose of 1 mg. of Synkavite not only brought the prothrombin time to 6 seconds, which is normal, but also maintained it for 1 week. The greater absorbability of the water-soluble vitamin K preparations compensate for their somewhat lesser physiological potency as compared with the natural vitamin K_1.

Quantitative estimation of thromboplastic activity. MAURICE M. RAPPORT AND FRANK MALTANER.* *Div. of Labs. and Research, New York State Dept. of Health, Sloan-Kettering Inst. for Cancer Research, New York City.*

A sensitive and reproducible estimation of thromboplastic activity has been obtained employing a 2-stage method. Oxalated thromboplastic-free rabbit plasma is prepared using paraffined glass. The prothrombin solution is obtained by defibrination after recalcification with 4 volumes of 0.004 M $CaCl_2$ in saline. The fibrinogen source is prepared by adding 4 volumes of 0.02 M sodium oxalate in saline to the original oxalated plasma. One tenth of a milliliter of prothrombin solution, 0.1 ml. of 0.01 M $CaCl_2$ in saline, and 0.4 ml. of saline containing varying amounts of thromboplastin are incubated for 6 minutes at 37°C. Then 0.1 ml. of the 'dioxalated plasma' is added, and the time of clotting observed by a tube-tilting technic at 37°C. The activity is evaluated by determining the dependence of clotting time on quantity of thromboplastin for the region where the time increases with decreasing thromboplastin concentration. Unit activity is defined as the quantity of material which gives half the maximum activity (double the minimum clotting time). Variations in the prothrombin solution are controlled by employing as reference standard a stable phosphatidyl serine fraction, which shows unit activity in the range 0.13–0.24 μg. and is assigned an activity of 5000 units/mg. By this method beef lung thromboplastin prepared according to Chargaff has an activity of 2200 PS units/mg.; fresh acetone-dried rabbit brain yields 200 PS units/mg.; and rabbit vitreous humor contains 30 PS units/ml. (Supported in part by a cancer control grant from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.)

Effect of growth hormone on glucose oxidation. LILLIAN RECANT (introduced by CARL F. CORI). *Dept. of Biochemistry. Washington Univ. School of Medicine, St. Louis, Mo.*

Pituitary preparations on injection into intact rats have been shown to prevent the rise in respiratory quotient (R.Q.) associated with glucose administration (GREAVES *et al. J. Biol. Chem.* 133: 243, 1940). Crystalline growth hormone in amounts ranging upward from 30 gamma/100 gm. of body weight, injected 1 hour prior to glucose administration, produces the same effect. The mechanism of this action is being studied in the isolated rat diaphragm. In the presence of glucose, pyruvate or acetate, the R.Q. of the diaphragm from a fasted rat is increased from .7 to values approaching theory. The *in vitro* addition of growth hormone (25–100 gamma/ml. of medium) prevents the rise in R.Q. in the case of glucose but has no effect upon the pyruvate or acetate R.Q. This R.Q. depressing effect is not reversed by insulin *in vitro*. Measurement of $C^{14}O_2$ derived from C^{14} labeled glucose and pyruvate confirm the fact that the oxidation of glucose but not of pyruvate is inhibited by the growth hormone. Since the glucose uptake by diaphragm is not diminished, it appears that the growth hormone exerts a blocking action between hexose-monophosphate and pyruvate.

Rates of absorption of barbiturates in the rat. D. V. N. REDDY* AND LEOPOLD R. CERECEDO. *Dept. of Biochemistry, Fordham Univ., New York City.*

The absorption rates of 3 barbiturates, barbital-Na, amytal-Na and phenobarbital-Na, from the intestine of rats of the Sherman strain have been investigated. All 3 compounds were fed in water solution by stomach tube to adult rats which had been fasted for 14–16 hours at a level of 120 mg/kg. body weight. The animals were sacrificed at the end of 3 hours, the stomach and small intestines removed, and unabsorbed barbiturates determined spectrophotometrically. At the level fed, the females were found to be more susceptible than the males. The absorption rates of the 3 compounds expressed as milligrams absorbed per kilogram body weight per hour were as follows: barbital-Na 33.5 ± 2.04; amytal-Na 23.3 ± 0.28; phenobarbital-Na 25.0 ± 1.48. The faster rate of absorption of barbital, a long-acting barbiturate with a slow onset of action, as compared with that of amytal, a medium acting compound which acts almost immediately, suggests that the pharmacological effect of these compounds is not related to the rates of absorption.

Nature of multiple forms of lipoic acid. LESTER J. REED (introduced by ROGER J. WILLIAMS). *Dept. of Chemistry, Univ. of Texas, Austin.*

Several distinct compounds possessing acetate-replacing and pyruvate oxidase factor activity for certain lactic acid bacteria exist in biological preparations (REED *et al. J. Biol. Chem.* 192: 851, 1951; GUNSALUS *et al., J. Biol. Chem.*, in press). One of these substances has been isolated in crystalline form from processed liver and has been named α-lipoic acid (REED, DEBUSK, GUNSALUS AND HORNBERGER, *Science* 114: 93, 1951). Analytical data reveal that α-lipoic acid is a monocarboxylic acid possessing the formula $C_8H_{14}S_2O_2$ (REED, DEBUSK, GUNSALUS AND SCHNAKENBERG, *J. Am. Chem. Soc.*, in press). Sulfur is present in the form of a disulfide linkage. Treatment of α-lipoic acid with thiols, followed by oxidation with iodine, results in the formation of a series of new compounds possessing acetate-replacing factor activity. These substances appear to be mixed disulfides. Acid hydrolysates of natural materials contain, in addition to α-lipoic acid, a chemically related acidic substance which is more polar than α-lipoic acid. Treatment of the latter with oxidizing agents converts it to the more polar substance. Furthermore, the more polar acid is converted to α-lipoic acid by treatment with hydriodic acid or glutathione. It would appear that the more polar acid is an oxidation product of α-lipoic acid, probably a sulfoxide. The occurrence in biological preparations of mixed disulfides of α-lipoic acid and oxidation products thereof would explain in part the multiplicity of acetate-replacing and pyruvate oxidase factors observed previously.

Dinitrophenylhydrazones of steroid ketones. HANS REICH AND KEITH F. CRANE (introduced by LEO T. SAMUELS). *Dept. of Biochemistry, Univ. of Utah College of Medicine, Salt Lake City.*

Dinitrophenylhydrazones can be used for the characterization of steroid ketones, since they exhibit typical ultraviolet absorption spectra. A maximum at 390 mμ seems to be specific for monodinitrophenylhydrazones of Δ^4-3-ketosteroids, while the maxima of dinitrophenylhydrazones of other α,β-unsaturated ketones are shifted to shorter wave lengths (Δ^{16}-20-keto: 385 mμ, Δ^5-7-keto: 385 mμ, Δ^9-12-keto: 385 mμ, Δ^4-6-keto: 376 mμ). The bisdinitrophenylhydrazones of steroids with one saturated and one α,β-unsaturated keto group display a maximum at 380 mμ (androstenedione, progesterone). A keto group in 11-position and a hydroxy group in 17-position cause shift to shorter wave lengths (adrenosterone: 375 mμ, 11-ketoprogesterone: 375 mμ, 17-hydroxyprogesterone: 376 mμ). The influence of the 17-hydroxy group could also be shown with saturated ketones, e.g. pregnenolone: 367 mμ, allopregnane-3β,17α-diol-20-one: 362 mμ. All spectra were taken in chloroform. The dinitrophenylhydrazones were chromatographed on aluminum oxide and eluted with mixtures of hexane and benzene, benzene, or mixtures of benzene and chloroform, depending on their polarity. In some

cases complete separation of two closely related dinitrophenylhydrazones could be accomplished, for instance, progesterone bisdinitrophenylhydrazone and pregnenolone dinitrophenylhydrazone, androsterone acetate and testosterone acetate dinitrophenylhydrazones. Progesterone bisdinitrophenylhydrazone is eluted from the column before androstenedione bisdinitrophenylhydrazone, but the separation is not complete. The dinitrophenylhydrazones of pregnenolone and Δ^5,16-pregnadiene-3β-ol-20-one could not be separated thus far.

Pyridine nucleotide concentrations in human red blood cells. JOHN G. REINHOLD, ALICE F. HOGANS* AND JAMES R. STUART.* *Pepper Lab. of Clin. Medicine, Hosp. of the Univ. of Pennsylvania, Philadelphia.*

Pyridine nucleotide concentrations have been determined in red blood cells of healthy subjects and also in the presence of hepatic and renal insufficiency. The acetone condensation method of Levitas *et al.* (*J. Biol. Chem.* 167: 169, 1947) was used for determining total pyridine nucleotides, with modifications enabling more accurate measurement at high concentrations. Diphosphopyridine nucleotide (DPN) was measured in the same specimens by means of alcohol dehydrogenase prepared by the method of Racker (*J. Biol. Chem.* 184: 311, 1950). Almost all of the pyridine nucleotide was accounted for as DPN; however, small but consistent differences between total pyridine nucleotide concentrations and that of DPN suggested the presence of other pyridine nucleotides. Extremely high concentrations of pyridine nucleotides in red blood cells of patients ill with terminal liver disease have been reported from this laboratory previously (Reinhold and Clark, Boston Meeting, American Chemical Society, 1950). That this increase actually represents DPN was confirmed by application of the dehydrogenase method. The occurrence of such extremely high values appears related to parenteral administration of nicotinamide when there is marked renal insufficiency. It is plausible to assume that high concentrations of nicotinamide may exist in the blood under these conditions, thus favoring the synthesis of, while exerting protective action upon, the DPN. (This work was supported by funds granted by the Department of the Army on recommendation of the Commission on Liver Disease of the Armed Forces Epidemiological Board.)

Effect on liver lipids and serum cholesterol of various intakes of cholesterol and choline. JESSIE H. RIDOUT,* COLIN C. LUCAS, JEAN M. PATTERSON* AND CHARLES H. BEST. *Banting and Best Dept. of Med. Research, Univ. of Toronto, Toronto, Canada.*

In spite of the volume of work done on the results of ingesting cholesterol, many gaps exist in our knowledge of the effects of different dietary intakes of this component, and particularly concerning the influence of supplementary choline on the nature and concentration of the cholesterol in the blood serum under these different conditions. Accordingly, groups of 10 rats (Wistar strain, 100–125 gm.) were fed a series of diets containing cholesterol (0, 0.2, 0.8 and 1.6% respectively); to some of these diets supplementary choline chloride was added (0.092, 0.184, 0.368, 0.736 and 1.472%, respectively). The basal hypolipotropic diet contained casein 8, gelatin 6, soybean protein (Glidden α-protein) 6, salts 3, celluflour 2, sucrose 63, beef fat 10, corn oil 2, with 13 vitamins. In the absence of choline, only a moderate increase in the serum cholesterol occurred when diets containing cholesterol were ingested, but the presence of choline caused large increases in the serum cholesterol. Data showing the extent to which dietary cholesterol and choline affect the liver lipids and serum cholesterol in preventative experiments will be presented. The time relationships of the curative action of choline on the cholesterol fatty liver, and serum cholesterol values under these conditions, will also be given.

Sulfhydryl groups and heme-heme interaction in hemoglobin. AUSTEN F. RIGGS (introduced by EDWARD H. FRIEDEN). *Biological Labs., Harvard Univ., Cambridge, Mass.*

With techniques described earlier (*J. Gen. Physiol.* 35: 23, 1951) the effects of dialysis and of *p*-chloromercuribenzoate upon the oxygen equilibrium of human hemoglobin have been examined. All measurements were made in borate buffer, pH 8.5. Fresh hemoglobin possesses a symmetrical oxygen equilibrium curve (fraction oxygenated vs. log oxygen pressure) with the value of n in Hill's equation about 2.9. Storage of hemoglobin at pH 8.5 for 12 hours at 5°C. does not change n. Dialysis, however, at the same pH and temperature, results in a symmetrical curve with n about 2.0. Addition of the sulfhydryl poison, *p*-chloromercuribenzoate, (1.8×10^{-3}M) to such a dialyzed solution produces a further drop in n to about 1.4. These changes are in large part reversed on the addition of glutathione. The value of n in Hill's equation is a measure of heme-heme interaction, so these observations mean that dialysis at pH 8.5 greatly decreases the interaction, and that *p*-chloromercuribenzoate almost completely blocks it. It is therefore concluded that sulfhydryl groups play a large part in the mechanism of heme-heme interaction. The relation of these to other observations—the effects of formaldehyde (Guthe and Wyman, 1951–52), and urea

(Wyman, 1948), and certain phenomena connected with sickle cells (Stetson and Chandler, 1948)—is discussed. (This research was supported in part by the Medical Sciences Division of the Office of Naval Research.)

Concentrative uptake of amino acids by Ehrlich mouse-ascites carcinoma cell. T. R. RIGGS,* H. FISCHER,* I. M. PALATINE,* G. S. WELLS* AND H. N. CHRISTENSEN. *Tufts College Med. School, Boston, Mass.*

The cells were shaken 1–1.5 hours in Krebs' Ringer-bicarbonate solution (or in ascitic fluid) at 37°, with the test amino acid usually at 5 mM. Upon chemical analysis the amino acid was always found concentrated in the cell, in the following order of decreasing activity: glutamate, glycine > alanine > histidine > citrulline > hydroxyproline > proline, lysine > ornithine, arginine, phenylalanine > valine, isoleucine, leucine. The D-isomers were also concentrated, but to a smaller extent. The amino acid was recovered practically quantitatively, the following showing losses: arginine (ornithine and urea formed), glutamate, glutamine (alanine formed), serine (glycine formed), ornithine (alanine formed but no α-amino acid N lost). Generally concentration was stronger from Krebs' medium than from ascitic fluid. Adding a second amino acid at 10 mM inhibited uptake of glycine (2 mM), the following order extending for nearly complete to slight inhibition: methionine, alanine, proline, glutamine, asparagine, hydroxyproline, threonine, leucine, histidine, valine, isoleucine. The D-isomers were less inhibitory. The order was almost the same when alanine, phenylalanine or histidine were tested. Histidine appeared to be concentrated as a neutral zwitterion as indicated by the relation of activity to pH. Anionic amino acids were without effect; the cations arginine and lysine were uniformly stimulatory to accumulation, except when a cationic amino acid was under test. Bound glycine or alanine were not detected when their uptake was inhibited by another amino acid, in contrast to Gale's finding of cysteinylglutamate accumulation when glutamate uptake by *S. aureus* was inhibited by cysteine.

Inhibition of bacterial and brain glutamic acid decarboxylases. EUGENE ROBERTS. *Wernse Lab. of Cancer Research, Washington Univ. School of Medicine, St. Louis, Mo.*

A series of compounds structurally related to glutamic acid (obtained through the courtesy of Dr. Karl Pfister of the Research Laboratories of Merck and Co., Inc.) was tested for the inhibition of the glutamic acid decarboxylase activity of acetone powders of mouse brain and of suitably grown *E. coli*. DL-α-Methylglutamic acid was the most potent competitive inhibitor found for the bacterial enzyme, the K_I (inhibitor-enzyme dissociation constant) being 2.69×10^{-4} as compared to the K_s (substrate-enzyme dissociation constant) of 2.69×10^{-3}. This compound was also the most effective one in inhibiting the utilization of glutamic acid for growth by *L. arabinosus*. The diastereoisomeric racemates of β-hydroxyglutamic acid, methionine sulfoxide, various N-substituted γ-amides of glutamic acid, and compounds related to pyrrolidone carboxylic acid were not inhibitory for the bacterial enzyme in the concentrations tested. α-Methylglutamic acid was only a weak competitive inhibitor of the brain enzyme ($K_I = 1.1 \times 10^{-2}$; $K_s = 5.9 \times 10^{-3}$). Although derivatives of semicarbazide and hydroxylamine were inhibitors of the decarboxylase from both sources, none of them was more effective than the parent compounds themselves. The latter compounds are inhibitory because of their reactivity with pyridoxal phosphate, the coenzyme for the decarboxylases. Studies of the mode of this inhibition will be discussed. Evidence of the *in vivo* antagonism of α-methylglutamic acid to glutamic acid also was obtained when it was found that this compound significantly enhanced the incidence of audiogenic seizures in a susceptible strain of mice tested under standard conditions. Marked reduction in seizures occurred when glutamic acid was given.

Influence of adrenal cortex on protein transformations in vitro. SIDNEY ROBERTS. *Dept. of Physiological Chemistry, Univ. of California School of Medicine, Los Angeles.*

It has been previously noted that incubation of surviving rat liver minces in a serum medium resulted in a disappearance of albumin and an increase in the α-globulin fraction, as measured electrophoretically (*J. Biol. Chem.* 180: 505, 1949). Lymphoid tissues, including spleen, released a protein which travels with the electric mobility of β-globulin; again, serum albumin disappeared concurrently. In an extension of these studies, it has been found that these transformations appear to be regulated by the level of adrenal cortical secretions in the animals from which the tissues have been obtained. Adrenalectomy depressed the utilization of serum albumin by all tissues studied. Previous administration of cortisone or ACTH accentuated the transformations noted. The net release of protein by surviving tissues to the serum medium was likewise directly correlated with the level of adrenal cortical activity in the animal supplying the tissues. No such correlation was noted when the tissues were incubated in a phosphate buffer medium. In the latter medium, the proteins released were several in nature and resembled aqueous extracts of the tissues, when examined electrophoretically.

Incorporation of cytidine into nucleic acids. IRWIN A. ROSE* AND B. S. SCHWEIGERT. *Div. of Biochemistry and Nutrition, American Meat Inst. Fndn. and Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.*

Randomly labelled cytidine was prepared by growing *Euglena gracilis* on $C^{14}O_2$. *E. coli* grown in the presence of the labelled cytidine incorporated the base to about the same extent into RNA cytosine and uracil. The ribose of the corresponding ribosides was only about 10% as active as the pyrimidines and of the same activity as the ribose of the purine ribosides. DNA thymine and cytosine were also active. Cytosine was incorporated to a similar extent and with like pattern as the base of cytidine indicating little or no incorporation of the intact nucleoside. Since the rat does not utilize free pyrimidines for nucleic acid synthesis it was of interest to compare the rat with *E. coli*. Unlike the latter, RNA cytidine of the rat was equally labelled in the base and in the ribose when cytidine was injected. However, the uracil of the uridine was more active than the ribose of uridine. The purine ribosides were not active. The desoxyribose of thymidine and desoxycytidine was also radioactive indicating conversion from the ribose of cytidine. In all cases base and sugar were counted separately by first exchanging the base of the nucleoside with hypoxanthine with the nucleosidase of *E. coli* followed by the isolation of inosine or desoxyinosine. Desoxyribosides were freed of riboside contamination by paper chromatography using a borate-butanol system.

Biosynthesis of hyaluronic acid by *Streptococcus hemolyticus*. SAUL ROSEMAN,* FRANCES E. MOSES,* ROBERT ABELES,* JULIO LUDOWIEG* AND ALBERT DORFMAN. *Depts. of Pediatrics and Biochemistry, Univ. of Chicago, Chicago, Ill.*

The mechanism of synthesis of mucopolysaccharides is unknown. This report is concerned with the biosynthesis of hyaluronic acid by a strain of Group A streptococcus. D-glucose-1-C^{14} was incorporated into a semisynthetic medium (Bernheimer, A. W. and Pappenheimer, A. M., J. Bact. 43, 481, 1942) and after growth of the organism, hyaluronic acid was isolated from the supernatant medium utilizing phosphomolybdic acid as a protein precipitant. The hyaluronic acid was hydrolyzed and the CO_2 (which represents the C_6 of glucuronic acid) liberated and collected. Glucosamine hydrochloride was isolated from the hydrolysate by treatment with Dowex 1, followed by conversion to the hydroxy-naphthaldehyde Schiff base (Z. E. Jolles, and W. T. J. Morgan, *Biochem. J.* 34: 1184, 1940) and partition chromatography of the base on silica gel. Suitable control experiments demonstrated that this procedure yields glucosamine hydrochloride free from trace amino acid contamination. A method was developed for the degradation of glucosamine consisting of oxidation to glucosaminic acid and treatment with ninhydrin or chloramine-T to yield CO_2 (from C_1) and arabinose. The results obtained indicated that the major fraction of the radioactivity of the glucosamine was present in C-1. These data suggest that the glucosamine moiety of the hyaluronic acid arises from glucose. (This investigation was supported by a grant from the United States Public Health Service.)

Preparation of purified human plasma fibrinogen by a cold ether procedure. LOUIS ROSENFELD* AND GEORGE Y. SHINOWARA. *Depts. of Physiological Chemistry and Pathology, Ohio State Univ., Columbus, Ohio.*

From reconstituted normal human dried plasma is obtained Fraction I (Cohn), 5.0–5.8 g/l., of which 60–65% is thrombin-clottable protein. A 2% suspension in citrate-phosphate buffer (pH, 7.2; r/2, 0.129) is cooled to 0°C and centrifuged. An acetate buffer is added to the supernatant to pH 5.0 at 0° to −1°C. Cold ethyl ether to a final concentration of 5% is then added resulting in a precipitate (A) containing 84–85% fibrinogen with a yield of 71–78%. This fraction (A) is reconstituted to its original volume with citrate-phosphate buffer and reprecipitated at pH 7.2 ± 0.2 with cold ether at 5% concentration. This precipitate (B) has a purity of 92–95% fibrinogen and a yield of 35–45%. Repeating the last step results in a preparation (C) of 97% purity and a yield of 25–30%. All yields are based on the fibrinogen concentration in the original Fraction I solution. The properties of the highly purified fibrinogen will be presented.

Distribution of cyanide-sensitive alkaline phosphatase activity in normal and regenerating rat liver. OTTO ROSENTHAL, BEATRICE G. NOVACK* AND JUNE W. BRODIE.* *Harrison Dept. of Surgical Research, Univ. of Pennsylvania Schools of Medicine, Philadelphia.*

We have previously reported that the striking increase in alkaline phosphatase activity of regenerating liver was mainly due to a cyanide-sensitive, magnesium-insensitive component, which rose from a normal mean of 28% of total activity to 74% during the first day after 70% hepatectomy. To explore the localization of this component, normal livers, as well as surgically reduced livers at one day after partial hepatectomy, were perfused *in situ* with isotonic sucrose solution and homogenized in this medium. The homogenate (H) was then separated by centrifugation into nuclear fraction (N_w), mitochondrial fraction (M_{w2}) and supernatant (S_1), according

to the method of Schneider and Hogeboom. The cyanide-sensitive phosphatase activity in H, N_w, M_{w2} and S_1 amounted to 25, 70, 42 and 21, and 61, 86, 81 and 50% of total activity in normal and regenerating liver, respectively. Corresponding figures for total phosphatase activity per gm. of nitrogen, expressed as relative concentrations, were 1.0, 0.6, 0.4, 1.7, and 3.7, 11.2, 2.1 and 3.7. The data indicate that, in resting liver, the cyanide-sensitive phosphatase activity is localized mainly in the nuclear fraction and that this fraction exhibits the greatest increase in enzyme concentration during the early phase of liver regeneration. (This work was done under contract number DA-49-007-MD-143 between the University of Pennsylvania and the Department of the Army.)

Synthesis of biotin or a biotin analogue by the anaerobe, *Clostrodium perfringens* PB6K. EUGENE ROSENWASSER* AND M. J. BOYD. *Div. of Biological Chemistry, Hahnemann Med. College, Philadelphia, Pa.*

The anaerobic microorganism, *Clostridium perfringens* PB6K, will grow on a chemically defined medium (BOYD *et al.*, *J. Biol. Chem.* 174: 1948) provided biotin is present. Recent studies show that biotin may be replaced in the medium by the water soluble fatty acid esters of the polyoxyethylene derivative of sorbitan. Luxuriant growth of the microorganism is obtained, particularly, if lauric or oleic acid esters are present. Neutralized acid hydrolysates of the harvested bacterial cells and cell filtrates from media containing the mono-oleic acid ester, but no added biotin, were analyzed for biotin by using Cl. perfringens PB6K as the assay organism. Results of the assays indicate that biotin or a substance similar to biotin is formed in the cells and also appears in the cell filtrates. When the hydrolysates were treated with avidin, bacterial growth failed to occur, hence it was assumed that the free biotin or its analogue formed an avidin complex. Avidin did not nullify the growth stimulating effect of the oleic acid ester.

Incorporation of radioactive phosphate into certain protein-bound phosphorus fractions of brain. R. J. ROSSITER, H. A. DELUCA* AND K. P. STRICKLAND.* *Dept. of Biochemistry, Univ. of Western Ontario, London, Canada.*

The specific activities of the following protein-bound phosphorus fractions were determined in the brain of the cat after the intracisternal injection of radioactive phosphate: desoxypentosenucleic acid (I), pentosenucleic acid (II), both by the method of Hammarsten (*Acta Med. Scand.* 128: 634, 1947), an acid-insoluble fraction (III), an acid-soluble ester phosphate fraction (IV) and an inorganic phosphate fraction (V), all obtained by alkaline hydrolysis of the residue remaining after complete removal of the nucleic acids. The specific activities of these fractions were in the order V > IV > III > II > I. As was found by Davidson *et al.* (*Biochem. J.* 49: 311, 1951) for liver tissue, the specific activities of both the DNA (I) and the PNA (II) were considerably less than those of the corresponding fractions separated by the method of Schmidt and Thannhauser (*J. Biol. Chem.* 161: 83, 1945). The specific activity of the PNA (II) was also less than that of the Schmidt-Thannhauser PNA even after allowance had been made for the highly active inorganic phosphate (cf. V) and the non-nucleic acid ester phosphate (cf. IV) that appears as PNA in this procedure. Fraction III is present in low concentrations in brain, but Fraction IV, which had a specific activity more than three times that of PNA (II), accounts for about one-half of the protein-bound phosphorus of whole brain. This may represent the phosphorus of the inositol-containing lipid-protein complex recently described by Folch and LeBaron (*Federation Proc.* 10: 183, 1951). Whether the relatively high specific activity is genuine, or due to contamination with a much more active compound remains to be determined. The active Fraction V corresponds to the 'phosphoprotein' of Schmidt and Thannhauser.

Inhibitors and activators for ribonuclease and desoxyribonuclease. JAY S. ROTH (introduced by J. S. HEPBURN). *Div. of Biological Chemistry, Hahnemann Med. College, Philadelphia, Pa.*

The effect of various organic substances on the rate of degradation of yeast ribonucleic acid by pancreatic ribonuclease and desoxyribonucleic acid by pancreatic desoxyribonuclease has been studied using the turbidometric method of McCarty (M. MCCARTY, *J. Exptl. Med.* 88: 181, 1948) for both assays. Benzimidazole caused a marked increase in the rate of reaction of ribonuclease but had no effect on desoxyribonuclease. The test system consisted of 20.0 ml. of 0.2% RNA or DNA, 20.0 ml. of 0.17 M veronal-acetate buffer pH 7.75 (in DNAase assay 0.0075 M with respect to Mg^{++}) and 10.0 ml. of appropriate enzyme dilution. Aliquots were precipitated with acid at 2-minute intervals. The activating action of benzimidazole increased rapidly as the amount used increased from 0–50 mg. Greater quantities caused little further activation or showed a slight decrease. 2-Aminobenzimidazole was inhibitory and several other purine and pyrimidine derivatives had no effect. Heparin is a potent inhibitor of pancreatic ribonuclease, as little as 1 mg. added to the test system caused a significant reduction in activity. Heparin also inhibited pancreatic desoxyribonuclease. Using a different assay method based on the hydrolysis of radioactive

phosphate from phosphate labelled nucleic acid, (J. ROTH, *Biol. Bull.* 101, 212, 1951) heparin was found to inhibit other tissue ribonucleases. The effect of pH, Mg^{++} and salt concentration on the activating and inhibitory effects of these substances will be presented and the significance of the results, in terms of their bearing on the mechanism of cell division, discussed.

Metabolism of 1,5-C^{14}-glutaric acid. MORTON ROTHSTEIN (introduced by A. L. DOUNCE). *Dept. of Radiation Biol., Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

1,5-C^{14}-glutaric acid is actively metabolized in normal and diabetic rats. When phlorizinized animals were fed approximately 8 μc. of the acid (.21 μc/mg.), 50% of the radioactivity was recovered as expired carbon dioxide within 24 hours and 5% was recovered in the urine. About 2% of the fed C^{14} appeared in urinary glucose, the isotope being present only in carbons 3 and 4 of the molecule. Isolation of α-acetamino-γ-phenylbutyric acid of relatively high specific activity indicated the formation of a labeled two carbon fragment from the glutarate chain. Possible metabolic pathways to be considered include, *a*) the formation of butyrate by decarboxylation of glutarate, and b) β-oxidation of glutarate to acetonedicarboxylate which may yield acetoacetate. Intermediates such as carboxyl-labeled α-ketoglutarate and succinate appear unlikely as direct metabolites because they should yield unlabeled acetate.

Identification of phosphate esters in tissues by paper chromatography. GEORGE ROUSER* AND WILLIAM F. NEUMAN. *Depts. of Biochemistry and Radiation Biology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Chromatograms of rabbit tissue extracts were developed as previously described (*Federation Proc.* 10: 240, 1951). Solvents recommended by Hanes and Isherwood (*Nature* 164: 1107, 1949) were also used. The compounds were detected *a*) by spraying with ninhydrin, *b*) by autoradiography of P^{32} labeled compounds or *c*) spraying with Hanes and Isherwood molybdate mixture followed by drying at 85°C. for 20 minutes and reduction with 2% ascorbic acid in hydrochloric acid (0.05N) or 2% iodine–potassium iodide solution in water. The compounds were identified on mixed chromatograms of known and unknown compounds, one of which was labeled with P^{32} (*Federation Proc.* 10: 240, 1951). A radioautograph was made on X-ray film and the chromatogram sprayed as described. Exact superimposition of the spots on autograph and chromatogram is evidence of identity. The identification of ethanolamine and serine phosphates was accomplished by this method. Prolonged acid hydrolysis of these esters gave rise to ethanolamine and serine as detected by paper chromatography. A phospolipid containing complex moving with phenol and collidine solvent fronts, after isolation from paper chromatograms, was found to release on acid hydrolysis serine and ethanolamine phosphates. The substance appears to be a cephalin-peptide complex. The R_f values in phenol and collidinlutidine were: ethanolamine phosphate (0.27, 0.08), serine phosphate (0.04,0.08), free ortho phosphate (0.09,0.08). The latter two gave somewhat variable values. Radiophosphorus samples from Oak Ridge when chromatographed showed several spots other than that of free ortho phosphate. Boiling with nitric acid causes their disappearance. These spots represent about 5% of the total radioactivity.

Beta disturbance of electrophoretic patterns in disease. JOSEPH I. ROUTH AND W. D. PAUL.* *Depts. of Biochemistry and Medicine, State Univ. of Iowa College of Medicine, Iowa City.*

In electrophoretic patterns of the descending boundary of plasma or serum, the beta globulin component often contains a sharp spike extending upward. Early investigators in electrophoresis called this the beta disturbance, but for comparative purposes we prefer to consider it a sharp spike. Kelley *et al.* reported a decrease in length of this spike in patterns from poliomyelitis sera. Since this spike may be related to lipoprotein complexes, it was suggested that these complexes decreased in poliomyelitis. In recent studies on poliomyelitis we compared the decrease of the length of the beta spike in plasma and serum patterns with those from normal individuals. Assuming a normal beta spike to be as high as the albumin peak, we observed decreases in the spike similar to those reported by Kelley *et al.* Patterns from normal children (77 plasma, 19 serum) and adults (37 plasma, 24 serum) exhibited beta spikes considerably shorter than those from normal children (20 serum) reported by Kelley *et al.* The beta spikes of over 1100 plasma and 500 serum patterns from patients with poliomyelitis, diabetes, rheumatic fever, arthritis and liver disease were measured and compared. In most instances, the serum patterns exhibited a greater decrease in the length of the beta spike than the plasma patterns. No marked difference was observed in the length of the beta spike in patterns from normal individuals as compared to patternd from patients with various diseases.

Light scattering studies on dexosyribonucleic acid. JOHN W. ROWEN (introduced by LEON A. HEPPEL). *Natl. Insts. of Health, Bethesda, Md.*

In this study the double extrapolation method (*J. Chem. Physiol.* 16: 1099, 1948) is applied to the

scattering of light from solutions of sodium thymonucleate. In 20 determinations the dissymmetry values fell in the range of 3.3–3.9 with an average intrinsic dissymmetry of 3.6. This value indicates that the molecular configuration is intermediate between that of a rod and a stiff coil and is in agreement with the finding of B. Bunce and P. M. Doty (*Science* 112: 642, 1950). The sample studied had a molecular weight of 4.05 million ± 420,000 and a coil length of 4,150 ± 250 Å. The coil lengths were determined from the light scattering of solutions of DNA in $MgCl_2$, $CaCl_2$ and KCl of ionic strengths between 0 and 6.0. The results indicate that the extent of coiling caused by the ions is small—less than 30%. They do not support the hypothesis (*J. Biol. Chem.* 178: 1007, 1949) that the activating effect of magnesium ions in enzymatic depolymerization is caused by change in coiling of the substrate. The zero slopes obtained when $\frac{HC}{\tau}$ is plotted versus C in the region of low ionic strength and high nucleate concentrations are not consistent with the proposal that the nucleate is in the form of a three dimensional network gel. Molecular weights as high as 22 million were obtained on two other high viscosity preparations. These preparations have not been studied as extensively as the above mentioned preparation, and the observed molecular weights must, therefore, be regarded as tentative until the larger values are confirmed. The implications of the above results will be discussed.

Propanediol phosphate in acetone metabolism. HARRY RUDNEY (introduced by WARWICK SAKAMI). *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

Previous work (*Arch. Biochem.* 29: 231, 1950) indicated that 1,2-propanediol phosphate (PDP) might be an intermediate in the conversion of acetone methyl carbon to 'formate' and in the direct transformation of acetone to a three carbon precursor of the glycolytic cycle (*J. Biol. Chem.* 193: 199, 1951). These investigatons have been continued. Using P^{32} PDP as a tracer, a method has been devised for its isolation from liver. It has been found that most of the PDP resides in the alcohol-insoluble barium-soluble fraction (Le-Page fractionation scheme). C^{14} carbonyl labeled acetone was administered to fed rats, and the distribution of isotope in liver glycogen and PDP was determined. The concentration of isotope in the carbons of these compounds expressed as counts/min/mg. of carbon was as follows: PDP, 1:0, 2:550, 3:0; glucose, 1,6:98, 2,5:124, 3,4:123. These results support the postulate that acetone was converted to PDP. The labeling of the carbons of glucose can be explained on the basis of a split of PDP to give carboxyl labeled 'acetate,' combined with the direct conversion of PDP to a three carbon intermediate of the glycolytic cycle. Although these results are in accord with the initial hypothesis, that propanediol or its phosphate ester is an intermediate in acetone metabolism, they do not indicate whether PDP is a direct intermediate, or whether it is related to acetone metabolism merely by virtue of being a metabolite of some compound which is the active intermediate involved.

I^{131}-labelled Girard's reagent 'Iodo-P' as a tool in ketosteroid analysis. W. S. RULIFFSON, H. M. LANG AND J. P. HUMMEL (introduced by G. KALNITSKY). *Dept. of Biochemistry, State Univ. of Iowa, Iowa City.*

I^{131}-labelled acethydrazide-3-iodopyridinium bromide (Girard Iodo P) was synthesized from 3-iodopyridine I^{131} which was formed by exchange between NaI^{131} and 3-iodopyridine. The Girard Iodo-P reagent coupled readily under the usual conditions with steroids having keto-groups at positions 3, 11, 17 and 20 to form the typical water-soluble hydrazones. When subjected to ascending partition paper chromatography with butanol: water, the hydrazones of mono-ketonic steroids were readily separated from those of poly-ketonic steroids. The hydrazones of androsterone, isoandrosterone, dehydroisoandrosterone and testosterone, all having R_f values of about 0.60, were inseparable. The radioactivity on the paper was directly proportional to the amount of ketosteroid present. Recovery of microgram amounts of isoandrosterone by radio-activity measurements was essentially quantitative. Steroids with two ketonic groups formed chiefly the dihydrazone derivatives, although small amounts of the monohydrazones were always observed. The dihydrazones generally had R_f values of about 0.15–0.25 whereas the R_f values of the monohydrazones were 0.50–0.65. The use of paper treated with Quilon (Du Pont) did not greatly change the R_f values obtained with untreated paper but resulted in somewhat sharper zones.

Electrophoretic studies of proteins of normal and irradiated rabbit lenses. C. ORVIS RUPE (introduced by CHESTER J. FARMER). *Dept. of Biochemistry, Northwestern Univ. Med. School, Chicago, Ill.*

Electrophoretic studies were made of the proteins of normal and irradiated lenses of young adult rabbits. The right lenses received in situ 2000 r of x-rays, while the left lenses served as controls. At given intervals after irradiation, the animals were sacrificed and the lenses removed. The lenses were extracted separately with veronal buffer at pH 8.6 and the extracts used for the electrophoretic studies. The normal lenses showed four components, which made up 95% of the total

lens proteins. Component I contained approximately 32%, component II—48%, component III—10%, compound IV—6%, and the insoluble protein—5%. Component I had a mobility of (cm^2/volt/sec.) 6×10^{-5}, component II—3×10^{-5}, component III—1.5×10^{-5}, and component IV—1.2×10^{-5}. The lenses which had been irradiated did not differ in pattern, distribution or mobility from the control lenses, for at least 7 weeks after exposure to x-rays. This, however, is prior to any clinical manifestation of opacities. (Work performed under contract with the Atomic Energy Commission.)

Nature of isomerism in ribonucleotides. HENRY Z. SABLE (introduced by GERHARD SCHMIDT). *Dept. of Biochemistry and Nutrition, Tufts College Med. School, Boston, Mass.*

Cohn, Carter and Loring have shown that hydrolysates of ribonucleic acids contain isomeric adenylic, guanylic, cytidylic and uridylic acids, which are designated as A and B isomers. Most evidence favors positional isomerism involving the phosphate residue, but α- β-isomerism of the *N*-glycosidic bond has not been eliminated as a possibility. Adenylic acids A and B were dephosphorylated with prostate phosphatase, and then treated with adenosine deaminase. The rate of the deamination reaction was the same with both nucleosides. The adenylic acids were converted to inosine, and then treated with yeast nucleosidase. The rate of hydrolysis of the glycosidic bonds was identical. Cytidylic acids A and B were treated with prostatic phosphatase and then with cytidine deaminase of yeast or *E. coli*. The rate of deamination was the same with both nucleosides. These results favor the view that the A and B nucleotides yield the same nucleoside, and therefore the nucleotides are not α- β-anomers. A study of physical properties of the nucleosides is in progress.

Metabolism of isovaleric acid. WARWICK SAKAMI AND JEAN LAFAYE.* *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

The possibility that the isopropyl group of isovaleric acid (IVA) is metabolized through acetone or acetoacetic acid has been studied using 3-C^{14}- and 4,4′-C^{14}-IVA by comparing the tracer pattern in liver glycogen with that obtained with acetone and acetoacetate. Sakami and Lafaye (*J. Biol. Chem.* 193: 199, 1951) showed that $C^{14}H_3 \cdot CO \cdot C^{14}H_3$ yields glycogen with the C^{14} of carbons 1,6 > 2,5 whereas $C^{14} \cdot CO \cdot C^{14}H_2 \cdot COOH$ yields 1,6 = 2,5; $CH_3 \cdot C^{14}OCH_3$ yield 2,5 > 1,6 and $CH_3C^{14}O \cdot CH_2C^{14}OOH$ gives labeling almost entirely 3,4. The unequal labeling of 1,6 and 2,5 by acetone is believed to occur because of direct oxidation of part of the acetone to a C_3 intermediate of glycolysis. In addition $C^{14}H_3 \cdot COC^{14}H_3$ labels the liver serine and choline methyl groups apparently because of a second mechanism in which 'formate' is formed by a 2- and 1-carbon cleavage. Acetoacetate apparently is not metabolized via acetone but rather via a C_2 cleavage. The results with IVA when glucose was administered simultaneously were in agreement with those expected if the isopropyl group was converted to acetoacetate via carboxylation. Thus 3-C^{14}-IVA gave almost exclusively 3,4 labeling and 4,4′-C^{14}-IVA gave 1,6 = 2,5. However when 4,4′-C^{14}-IVA was administered together with glycine the C^{14} in the glycogen was 2,5 > 1,6 and the β carbon of serine and methyls of choline possessed low activity. This labeling of glycogen (2,5 > 1,6) by 4,4′-C^{14}-IVA is not explained by any of the proposed mechanisms and apparently under these conditions the isopropyl moiety follows in part some other pathway. The nature of this pathway is being further investigated.

Role of uroporphyrin III as a precursor of protoporphyrin IX. KURT SALOMON, JONAS E. RICHMOND AND KURT I. ALTMAN (introduced by WALTER R. BLOOR). *Depts. of Radiation Biology and Biochemistry, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

C^{14}-labeled uroporphyrin III was prepared in order to demonstrate the conversion of uroporphyrin III to protoporphyrin IX. For this purpose alpha-C^{14}-labeled glycine was administered to 2 rabbits and uroporphyrin subsequently isolated from the bone marrow of these animals. Inert potassium uroporphyrinate (1.8 gm.) was added to 19.5 gm. of bone marrow homogenate and labeled uroporphyrin was subsequently isolated and purified; 1.6 mg. of pure uroporphyrin octamethylester was thus obtained with an activity of 72 counts/min/mg. Then 1.2 mg. of pure C^{14}-labeled potassium uroporphyrin was incubated with a suspension of bone marrow cells for 20 hours. Protoporphyrin IX dimethylester and uroporphyrin octamethylester isolated from this mixture were of a purity of 96 and 99%, respectively. The C^{14}-activity of the protoporphyrin dimethylester was 20 counts/min/mg., no detectable activity was present in the uroporphyrin octamethylester. Since about half of the C^{14}-activity added as uroporphyrin was recovered in the protoporphyrin fraction, and since uroporphyrin had lost most of its activity at the end of the experiment, we conclude that uroporphyrin III is a precursor of protoporphyrin IX. (This work was performed under contract with the United States Atomic Energy Commission at the University of Rochester Atomic Energy Project, Rochester, New York.)

Role of coenzyme A and DPN in the oxidation of α-ketoglutarate. D. R. SANADI AND J. W. LITTLEFIELD (introduced by D. E. GREEN).

Inst. for Enzyme Research, Univ. of Wisconsin, Madison.

The α-ketoglutaric oxidase, homogeneous in the ultracentrifuge and electrophoresis, (Abst. XII International Chemical Conference, New York, Sept., 1951) catalyzes the following reaction: α-ketoglutaric acid + DPN + CoA → succinyl CoA + DPNH + CO_2 + H^+. In the study of the stoichiometry of the reaction, the formation of succinyl CoA has been demonstrated by *a*) formation of hydroxamic acid, *b*) disappearance of CoA (arsenolysis by transacetylase), *c*) disappearance of −SH (nitroprusside reaction), and *d*) liberation of CoA and disappearance of hydroxamic acid reaction by boiling the enzymatic digest for 2 minutes at neutral pH. Thus, CoA and DPN can be regarded as the prosthetic groups of the oxidase. The equilibrium of the reaction is far to the right, and the DPNH formation is equivalent to the CoA added, suggesting a possible use in CoA assay. In some preparations of CoA of equal purity, however, only part of the CoA seems to react under identical conditions. The significance of this difference in behavior will be discussed. The availability of CoA in gram quantities has made it possible to undertake the isolation of succinyl CoA from the digest mixture and to use succinyl CoA as a substrate for other enzymatic reactions. Similar results with pigeon breast pyruvic oxidase, implicating acetyl CoA as the first product of oxidation, have been obtained.

Effect of erythrocyte cholesterol on resistance to lysis by saponin. CURTIS N. SANDAGE AND YAYE FURUTANI (introduced by C. C. WANG). *Bacteriology Research Unit, Hines Hosp., Hines, Ill.*

Cholesterol in plasma is known to function as an anti-hemolytic agent against several lysins, including saponin. Results of work reported here show that cholesterol which is associated with the erythrocyte, however, rather than with plasma, not only does not protect the cell, but appears to enhance susceptibility to lysis by saponin. Erythrocytes containing abnormally high concentrations of cholesterol were thoroughly washed and exposed to serial dilutions of saponin. Following brief incubation periods, remaining cells were quickly removed from saponin, washed, and their cholesterol content was determined. Cells recovered from very low saponin concentrations contained a high content of cholesterol, whereas cells recovered from saponin concentrations sufficient to cause a high degree of lysis were found to have a low cholesterol content. Intermediate saponin concentrations yielded intermediate cholesterol values; thus, a direct correlation appears to exist between the cholesterol content of red cells and their susceptibility to saponin. The following observations were also made: *1*) a general direct correlation exists between red cell cholesterol and mean corpuscular volume, and *2*) the cholesterol content of erythrocyte suspensions decreased following storage under refrigeration, suggesting that those cells which contained most cholesterol were least stable under these conditions.

Studies on amino acid imbalances in the rat. H. E. SAUBERLICH. *Dept. of Animal Husbandry and Nutrition, Alabama Polytechnic Inst., Auburn.*

Studies were made with weanling rats on various amino acid imbalances, including tryptophan, methionine, lysine, and isoleucine. Diets were devised in each case to furnish suboptimal amounts of the amino acid under study. This was accomplished by the use of diets containing low levels of casein or peanut meal and supplemented with the appropriate amino acids to obtain a condition whereby the amino acid under study was limiting. Such diets permitted fair to normal growth. Upon the addition to these diets of proteins such as oxidized casein, gelatine, zein and hemoglobin, imbalances in tryptophan, methionine, lysine, and isoleucine could be produced. Such imbalances resulted in depressed growth, which could be corrected by the addition of the corresponding amino acid to the diet. For example, growth on a 10% casein diet supplemented with cystine and niacin was reduced from 26.5 gm/week gain to 6.0 gm/week upon the addition of 20% oxidized casein supplemented with cystine and methionine. An addition of 0.1–0.15% of L-tryptophan to this diet was required in order to obtain normal growth. The imbalance conditions appeared to be associated with an increased urinary excretion and low blood levels of the limiting amino acids. In many instances, the livers of the animals appeared fatty. Values for the amino acid and fat analyses will be reported.

Paper chromatographic separation of C_{19} and C_{21} ketosteroids. KENNETH SAVARD (introduced by RALPH I. DORFMAN). *Worcester Fndn. for Exptl. Biology, Shrewsbury, Mass.*

Modifications of the original technique of Zaffaroni, Burton and Keutman (1950) have been studied using propylene glycol as the preferred stationary solvent. Steroid mobilities increase with rising polarity of such mobile solvents as hexane, ligroin, benzene and toluene. The system ligroin-propylene glycol has the widest utility in both C_{19} and C_{21} series. The following relationships of chromatographic mobilities exist: 3-ketosteroids > 3,17- or 20-diketosteroids > 3,11, 17- or 20-triketosteroids; 3-ketosteroids > Δ^4-unsaturated 3-ketosteroids > $\Delta^{1,4}$-diene-3-ketosteroids; Monohydroxysteroids > dihydroxysteroids > trihydroxysteroids. In the C_{19} series

the retardation effect of 1 hydroxy group is roughly equivalent to that of 2 keto groups. In the C_{21} series the same relationship is true, although the hydroxy group at position 17 has greater retarding effect than one located at positions 11 or 21. Physical methods employed in locating the positions of steroids on developed chromatograms include *a*) direct measurement of ultraviolet absorption of the paper strip in the Beckmann Spectrophotometer by means of an adapter designed by Tennent (1951), *b*) fluorescence of phosphoric acid-treated paper strips in ultraviolet light (Neher and Wettstein, 1951), *c*) radioactivity of areas of chromatograms, in the separation of C-14 labeled steroid mixtures. Chemical tests include *a*) dinitrophenylhydrazine (saturated ketones and α,β-unsaturated ketones), *b*) aqueous osmic acid (α,β-unsaturated ketones), *c*) aqueous selenious acid (Δ^5 and Δ^7 steroids) and *d*) modification of the Zimmerman reaction (17-ketosteroids). Application of these methods to known mixtures of steroids and to unknown mixtures obtained in the perfusion of C-14 labeled acetate through isolated human testes will be presented.

Serine phosphoric acid from diisopropylphosphorylated chymotrypsin. NORWOOD K. SCHAFFER,* STEPHEN C. MAY, JR.* AND WILLIAM H. SUMMERSON. *Enzyme Chemistry Branch, Chemical Corps Med. Labs., Army Chemical Center, Md.*

Diisopropyl fluorophosphate (DFP) is known to inactivate the enzyme chymotrypsin, with the introduction of a diisopropylphosphoryl group into the enzyme molecule. To obtain some information regarding location of this new group in the protein, we reacted chymotrypsin with DFP labeled with radioactive phosphorus, isolated and purified the reaction product, and subjected it to partial hydrolysis by enzymes and acid. The hydrolysate was fractionated on a hydrogen form Dowex-50 column, with 0.05 N hydrochloric acid as the running solvent. Two major radioactive fractions were obtained. The first fraction contained about 50% of the original radioactivity and consisted almost entirely of inorganic phosphate, presumably produced by acid hydrolysis of all organic groups originally attached to phosphorus. The second major fraction, obtained in about 30% yield, contained a single ninhydrin-reacting component associated with the radioactive phosphorus. Paper chromatography indicated that this material might be serine phosphoric acid. Synthetic serine phosphoric acid added to the radioactive material was inseparable from the latter by paper and column chromatography, diffusion through aiporous diaphragm, ionophoresis, and fractional recrystallization. Microbiological assay of the radioactive material after prolonged acid hydrolysis proved serine to be present in equimolar amount to phosphorus. Control experiments demonstrated that under conditions where mild hydrolysis does not destroy serine phosphoric acid, the isopropyl groups of DFP are split from the DFP phosphorus. It is concluded that the ultimate, but not necessarily the immediate, site of attachment of DFP to chymotrypsin is through an OH group of one of the serine moieties of the enzyme.

Metabolism of histamine-2-C^{14}-imidazole. RICHARD W. SCHAYER (introduced by G. L. FOSTER). *Rheumatic Fever Research Inst., Northwestern Univ. Med. School, Chicago, Ill.*

L-Histidine-2-C^{14}-imidazole, synthesized by modifications of the method of Ashley and Harington (*J. Chem. Soc.* 2586, 1930) was enzymatically decarboxylated producing histamine-2-C^{14}-imidazole which was isolated and purified as the dipicrate. The activity was 9.3×10^6 cpm/mg. histamine base. Histamine was administered to animals as the dihydrochloride in physiological quantities. After 20 hours all radioactivity was excreted in urine; expired carbon dioxide was negative. Paper chromatograms of rat and guinea pig urine showed 3 radioactive peaks which remained unchanged after acid hydrolysis. Isotope dilution revealed small amounts of histamine and acetyl histamine in rat and guinea pig urine. The presence of imidazole acetic acid could not be conclusively demonstrated and, if present, contributes less than 2–3% of the total urinary radioactivity. Evidence will be presented to suggest that the unidentified urinary metabolites of histamine are not conjugates nor are they formed from histamine by simple alteration of the side chain. It is proposed that these compounds have undergone some modification of the imidazole ring but possess stability sufficient to prevent the oxidation of the 2-carbon to carbon dioxide.

Xanthine oxidase distribution in liver cell fractions. ARNOLD H. SCHEIN AND EMILY M. YOUNG (introduced by KURT G. STERN). *Dept. of Biochemistry, College of Medicine, Univ. of Vermont, Burlington.*

The xanthine oxidase (X.O.) activity of fowl and rodent liver cell fractions isolated by differential centrifugation (Hogeboom and Schneider) was assayed by spectrophotometric and manometric techniques. Correlation of results by spectrophotometry and manometry was obtained when corrections were made for nonoxidative disappearance of xanthine. The increase in absorption of the medium at λ 290 mμ (uric acid appearance) and the simultaneous decrease at λ 270 mμ (xanthine disappearance) served as an index of X.O. activity in fowl (uricase absent); only the

latter change in absorption may be employed for the same purpose in rodents (uricase present). Manometric assays of X.O. in mammalian liver were based on the total oxygen consumed in the reactions: *1*) xanthine + ½ O_2 → uric acid, *2*) uric acid + ½ O_2 → allantoin. Fractions in which the lack of uricase would be limiting were supplemented by the addition of this enzyme. When a purified, aged uricase preparation (X.O. free) from beef kidney was added to rodent liver supernatant fluid fractions, the X.O. activity was 80% that of the homogenates. X.O. activity of the mixed particulates (nuclear, mitochondrial and microsomal fractions) was negligible. In fowls, only the first reaction was measured. Here too, X.O. activity was found almost exclusively in the supernatant fluid fraction which contained approximately 100% of the homogenate X.O. activity. Addition of the activity of the mixed particulates brought recoveries up to 115%.

Observations supporting a debranching action of ribonuclease on yeast ribonucleic acid. GERHARD SCHMIDT, MARIA SERAYDARIAN,* KRIKOR SERAYDARIAN* AND S. J. THANNHAUSER. *Research Labs., Boston Dispensary, Tufts Med. School, Boston.*

Ribonucleic acid samples prepared from sodium chloride extracts of bakers' yeast according to a modification of G. Clarke and S. B. Schryver's procedure (*Biochem. J.* 11: 319, 1917) as well as purified samples of commercial ribonucleic acid, (Schwarz Laboratories) consume close to 100 equivalents of alkali/100 moles of total phosphoryl groups up to pH 8. A considerable part of the phosphoryl titration (between 18 and 25% of the total) occurs between pH 4.7 and pH 8 in agreement with observations of W. E. Fletcher, J. M. Gulland and D. O. Jordan (*J. Chem. Soc.* 33, 1946). This suggests triply esterified (or anhydrized) phosphoryls as branching points of the RNA molecules. Titration of digests obtained by exhaustive action of Kunitz's ribonuclease on RNA reveals the formation of new secondary as well as that of new primary phosphoryls by the enzyme action. The newly formed secondary phosphoryls are almost completely accounted for as pyrimidine mononucleotides, indicating their origin from cleaved terminal nucleotide groups of RNA. The newly formed primary phosphoryls must have originated from the hydrolysis of triply bound phosphoryls resulting in the debranching cleavage of the main polynucleotide chain to polynucleotides of smaller molecular weights. The latter effect might furnish an explanation for the initial volume dilatation observed during the action of ribonuclease by L. Vandendriesche in K. Linderstroem-Lang's laboratory (L. VANDENDRIESCHE. *Comptes rend. du Labatoire Carlsberg* 27: 341, 1951). The bearing of the observations on the structure of ribonucleic acid will be discussed.

Degradation of arginine by *Clostridium perfringens* (BP6K). GILBERT C. SCHMIDT,* MILAN A. LOGAN AND ALFRED A. TYTELL. *Dept. of Biological Chemistry, Univ. of Cincinnati, Cincinnati, Ohio.*

It has been shown that arginine is degraded by washed cell suspensions of *Clostridium perfringens* (BP6K), through the intermediate, citrulline, producing ammonia, carbon dioxide, and ornithine. Citrulline itself is attacked by washed cell suspensions of this organism with the production of ornithine, carbon dioxide, and ammonia. Citrulline was demonstrated as an intermediate by the colorimetric diacetyl reaction, by paper chromatography, and finally by isolation as the free base. Ornithine was demonstrated as an end product by the ninhydrin color reaction at pH 1, by paper chromatography, by enzymatic decarboxylation, and finally by isolation as the dibenzoyl derivative. Preliminary studies on the isolated citrulline indicate that it is the L(+) isomer. The following evidence is presented to indicate that the reaction proceeds according to the following scheme: arginine = citrulline + ammonia (*step a*): citrulline = ornithine + ammonia + carbon dioxide (*step b*). *1*) Citrulline has been isolated from the reaction mixture. *2*) It has been shown that the pH optimum for *reaction a* is different from the pH optimum for *reaction b*. *3*) Cell-free extracts of the enzyme for *reaction a* have been prepared completely free of enzyme (*b*) activity. *4*) In the presence of acetate buffer at pH 5.0 *reaction a* is not inhibited, but *reaction b* is inhibited from 60–100%. *5*) In balance sheet studies all the arginine originally present was accounted for as ornithine, carbon dioxide, ammonia, and citrulline.

Enzymic and chemical homogeneity of Schardinger dextrinogenase. SIGMUND SCHWIMMER. *Enzyme Research Div., Bureau of Agricultural and Industrial Chemistry, U. S. Dept. of Agriculture, Albany, Calif.*

Purified Schardinger dextrinogenase from *Bacillus macerans* was shown to consist of 1 component on the following evidence. The experimental maximum specific activity was close to that calculated by the method of Falconer and Taylor (*Biochem. J.* 40: 835, 1946) and was of the same order of magnitude found for other pure enzymes. Phase-rule solubility analysis showed about 90% of the protein present as one component. Preparations showed no trace of a closely related starch-hydrolyzing enzyme or other polysaccharide-degrading enzymes which were present in the impure preparations. Ultracentrifuge and conventional electrophoretic analyses showed the pres-

ence of only 1 component (single determinations). The paper electrophoresis technique of Kunkel and Tiselius (*J. Gen. Physiol.* 35: 89, 1951) revealed only 1 component at various pH values, salt and protein concentrations. The same technique showed the presence of 5 distinct proteins in partially purified preparations. Application of partition chromatography (MARTIN AND PORTER. *Biochem. J.* 49: 215, 1951), although resulting in extensive denaturation, revealed only 1 protein component of surviving activity. The first-order rate constant for the inactivation of the enzyme activity was identical with the constant for denaturation.

Preparation, properties, and assay of glucose-6-phosphate dehydrogenase and 6-phosphogluconate dehydrogenase of *E. coli*. D. B. MCNAIR SCOTT* AND SEYMOUR S. COHEN. *Children's Hosp. of Philadelphia and Dept. of Physiological Chemistry, Univ. of Pennsylvania, Philadelphia.*

Glucose-6-phosphate dehydrogenase (I) and 6-phosphogluconate dehydrogenase (II) have been extracted from *E. coli* grown in synthetic medium at 37°C. with glucose or gluconate as the sole carbon source under aerobic and anaerobic conditions. Methods were established for maximal extraction of the enzymes. I and II were separated and partially purified. The optimal temperature for both enzymes was 37°C. The activity of I was maximal over the pH range 7.6–8.6, and that of II from pH 6.6–7.6. The enzymes were activated maximally by divalent cations, Mg^{++} producing a sharp peak of activation at 0.015M for I and 0.02M for II. Calcium activation for II was maximal in a range of concentrations from 0.005M–0.08M. Inhibtion by various polyvalent cations and anions was also demonstrated. The Michaelis constants, Km, for I and II were 1.4×10^{-4}M/l. and 2.5×10^{-5} M/l., respectively. During exponential growth with optimal aeration in roller flasks, *E. coli* used glucose at about 5×10^7 molecules/cell/minute. Extracts from such bacteria reduced TPN in the presence of either glucose-6-phosphate or 6-phosphogluconate at a rate of 1.7×10^7 molecules TPN/cell/minute. The amounts of the 2 dehydrogenases thereby demonstrated were sufficient to account for about 30% of the bacterial consumption of glucose via the oxidative pathway of glucose-6-phosphate degradation. This value falls within the range of values obtained from studies of growth on C_1-labeled glucose (COHEN, S. S. NATURE 168: 746, 1951).

A triphosphopyridine nucleotide specific aldehyde dehydrogenase from yeast. J. E. SEEGMILLER (introduced by B. L. HORECKER). *Natl. Insts. of Health, Bethesda, Md.*

A diphosphopyridine nucleotide (DPN) linked aldehyde dehydrogenase has been prepared from liver by Racker (*J. Biol. Chem.* 177: 883, 1949). An aldehyde dehydrogenase preparation from yeast has been described by Black (*Arch. Biochem.* 34: 86, 1951) which is equally active with either DPN or triphosphopyridine nucleotide (TPN) and requires K^+ and cysteine as activators. Another aldehyde dehydrogenase has now been purified from fresh bakers' yeast (Fleischmann's) which reacts only with TPN and is activated by Mg^{++}, Ca^{++}, Ba^{++}, and Mn^{++}. After 80-fold purification by ammonium sulfate-pH fractionation, acetone precipitation and adsorption on aluminum hydroxide gel, the enzyme was free of alcohol dehydrogenase and the activity of various preparations with DPN was 260–700 times less than with TPN. Maximum activity was obtained with acetaldehyde as substrate although glycolaldehyde, proprionaldehyde and formaldehyde were also oxidized. Michaelis constants were 1.4×10^{-5} for TPN, 3.5×10^{-5} for acetaldehyde and 2.2×10^{-3} for glycolaldehyde. The reaction product formed in stoichiometric amounts was identified as acetic acid by Duclaux distillation. No evidence for the intermediate formation of acetyl-coenzyme A could be found.

Mechanism of action of 3-phosphoglyceraldehyde dehydrogenase. H. L. SEGAL (introduced by P. D. BOYER). *Div. of Agricultural Biochemistry, Univ. of Minnesota, St. Paul.*

Recent extension of the theory of enzyme kinetics (SEGAL, KACHMAR AND BOYER, Unpublished) involving 2 substrates shows that their K_m's must be equal if there is a compulsory combination of the substrates before combination with the enzyme. Such a mechanism has been proposed for the oxidative step of glycolysis which has a compulsory requirement for phosphate or arsenate (MEYERHOF AND OESPER. *J. Biol. Chem.* 170: 1, 1947). An alternate hypothesis suggests a combination of the aldehyde with sulfhydryl groups of the enzyme prior to reaction with phosphate or arsenate. Values of K_m for 3-phosphoglyceraldehyde and arsenate have been determined and found to be 3×10^{-4} moles/l. and 2×10^{-5} moles/l., respectively. The 15-fold difference in the values of K_m is strong evidence against the formation of an arsenylated aldehyde as the active form upon which the enzyme acts. An unlikely alternate possibility which would still allow such a combination is that one fifteenth of the total 3-phosphoglyceraldehyde exists in a different form and combines with the arsenate prior to oxidation. Determinations of the K_m of 3-phosphoglyceraldehyde at different levels of arsenic lead to the conclusion that there is not a compulsory combination of enzyme and triose before arsenate can combine if 'equilibrium' kinetics applies. Inhibition by high levels of arsenate has been observed. Important information about the possible

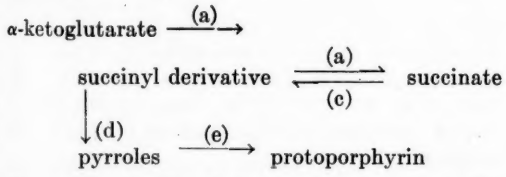
role of sulfhydryl groups of the enzyme may be obtained by studies of iodoacetate inhibition under various conditions. Preliminary experiments have shown that high levels of DPN and arsenate and moderate levels of triose fail to protect the enzyme.

Solubility of carbon monoxide in biological fluids. JULIUS SENDROY, JR. AND HAROLD A. COLLISON.* *Div. of Chemistry, Naval Med. Research Inst., Bethesda, Md.*

A study has been made of the various factors, temperature, pressure, and the composition of the fluid, which affect the solubility of carbon monoxide (uncombined) in plasma and whole blood. Determination of this value by the several methods which have been used in this work indicates an upward revision of 3.7% in the α (Bunsen solubility coefficient) for water currently in use, in the range of 20–40°C. The effect of salts, usually a decrease in α in most cases, has been determined for chloride, carbonate, bicarbonate, hydroxide, oxalate, citrate, lactate, and nitrite. From the values observed over a wide pH range, the specific ion effects may be calculated. The values for human plasma are 90.5% of those for water—a ratio within 1% of that previously found for both N_2 and O_2. Experiments on the determination of α in whole blood are in progress and will be reported. Preliminary work indicates that these values, likewise, will bear practically the same relationship (α blood: α H_2O = 1.00) to those for water found for other gases of physiological importance.

α-Ketoglutarate-succinate reaction; the formation of a succinyl intermediate from succinate. DAVID SHEMIN AND SELMA KUMIN.* *Dept. of Biochemistry, Columbia Univ., New York.*

We have concluded (SHEMIN AND WITTENBERG. *J. Biol. Chem.* 192: 315, 1951) that a succinyl derivative is utilized for protoporphyrin formation and that it arises from both ketoglutarate and succinate.



Here proof is offered that *reaction c* occurs. We have incubated duck erythrocytes with C^{14} carboxyl and C^{14} methylene labeled succinate and isolated labeled porphyrin. Theoretically carboxyl labeled succinate, on entering the citric acid cycle, cannot give rise to labeled porphyrin. If, however, *reaction c* occurs the protoporphyrin would be labeled from carboxyl labeled succinate and contain 40% of its radioactivity in the vinyl bearing pyrroles and 60% in the propionic acid bearing pyrroles. This predicted distribution of activity has been found. Moreover the presence of malonate influenced neither the activity nor the distribution of C^{14} in the porphyrin. Methylene labeled succinate should produce porphyrin of greater radioactivity than that from carboxyl labeled succinate since labeling can result from 2 pathways; *reaction c* and via the citric acid cycle. This was found experimentally. Furthermore, the addition of malonate now demonstrated inhibition in the utilization of methylene labeled succinate via the citric acid cycle producing a porphyrin with activity similar to that from carboxyl succinate. Addition of coenzyme A to the hemolyzed red cells increased the utilization of the succinates; the role of coenzyme A in this reaction will be discussed.

Influence of certain inhibitors on oxidation of citrate, isocitrate and cis-aconitate by rat liver slices. IRVING R. SHERMAN* AND RALPH C. CORLEY. *Dept. of Chemistry, Purdue Univ., Lafayette, Ind.*

The consumption of oxygen by slices of rat liver has been studied in the presence of citrate, DL-isocitrate and cis-aconitate (0.01 M), and the influence of sodium arsenite (0.0005 M), iodoacetate (0.01 M) and transaconitate (0.03 M) singly and together has been observed. The increase in the uptake of oxygen in the presence of the substrates was prevented or greatly reduced by the inhibitors, except when arsenite or transaconitate accompanied isocitrate. Arsenite or trans-aconitate inhibition was associated with decreased values for R. Q., iodoacetate, with increased values for R. Q., as compared to controls. Isocitrate yielded R. Q. values higher than for the other 2 substrates in the presence of iodoacetate plus trans-aconitate, but lower values in the presence of trans-aconitate. Keto acids other than pyruvic acid (presumably chiefly α-ketoglutaric acid) were estimated by a modification of the Friedeman-Haugen procedure, whereby pyruvate was removed by treatment with ammoniacal silver nitrate. Arsenite was accompanied by much more of such keto acids from citrate than from the other 2 substrates. Iodoacetate was accompanied by more of the keto acids from isocitrate than from the other 2 and the effect was accentuated in the presence of all 3 of the inhibitors. As the results obtained are not clearly interpretable according to the prevailing views of the metabolism of these compounds and the influences of these inhibitors, it is tentatively suggested that alternative mechanisms may occur or be called into play on occasion.

An anomalous effect of citrate on the respiration of rat liver slices in the presence of calcium ion. IRVING R. SHERMAN* AND RALPH C. CORLEY.

Dept. of Chemistry, Purdue Univ., Lafayette, Ind.

It is known that calcium ion represses respiration. With rat liver slices it has been found that the uptake of oxygen is less in the presence (Krebs-Henseleit solution) than in the absence of calcium ion. Although the consumption of oxygen is increased by 0.001 M citrate it is further depressed by 0.01 M citrate.

Biosynthesis of shikimic acid from labeled compounds. HAROLD SHIGEURA* AND DAVID B. SPRINSON. *Dept. of Biochemistry, Columbia Univ., New York City.*

In collaboration with Dr. Bernard D. Davis an investigation of the synthesis of shikimic acid (a 3,4,5-trihydroxycyclohexene-1-carboxylic acid) in *Escherichia coli* mutant 83-24 was carried out with the aid of various labeled precursors. In this mutant the synthesis of aromatic compounds (tyrosine, phenylalanine, tryptophane, *p*-aminobenzoic acid and *p*-hydroxybenzoic acid) is blocked by virtue of an inability to form them from shikimic acid. When grown on a glucose medium containing small amounts of these aromatic metabolites shikimic acid accumulates (DAVIS. *J. Biol. Chem.* 191: 315, 1951). Following the addition of $NaHC^{13}O_3$, $HC^{14}OONa$, and $C^{14}H_3C^{13}OONa$ to the medium the isolated shikimic acid contained only a small proportion of the added tracer. On the other hand, when 1-C^{14}-D-glucose was used an activity close to that of the precursor was obtained in the accumulated acid. (For similar results in the synthesis of tyrosine and phenylalanine in yeast, cf. GILVARG AND BLOCH. *J. Am. Chem. Soc.* 72: 5791, 1950). The distribution of activities in the various carbon atoms will be discussed.

Amino acid economy of a homofermentative organism. GERALD D. SHOCKMAN* AND GERRIT TOENNIES. *Inst. for Cancer Research and Lankenau Hosp. Research Inst., Philadelphia, Pa.*

Investigation of the fate of amino acids during the phase of logarithmic division of *S. faecalis* 9790 has revealed that, among the first 5 compounds studied, 4 (valine, threonine, histidine, lysine) are recovered in the bacterial substance and the medium without significant metabolic loss, while 1 (methionine) shows a metabolic loss of approximately 1 molecule for each 2 molecules assimilated. The fate of the methionine lost is being investigated by isotope and classical methods, with a view to its possible conversion to cystine, sulfate or unidentified sulfur compounds. With the possible exception of the methionine data, the initial findings thus support the hypothesis that amino acids are utilized per se and do not participate in energy production in this organism. If the normal carbohydrate metabolism of this organism is completely described by homofermentation, i.e. quantitative conversion of hexose to lactic acid, nonparticipation of amino acids in energy metabolism would have its counterpart in nonparticipation of carbohydrates in protein synthesis. It is the aim of the present project to verify these possibilities, because their correctness, i.e. a primordial character of both carbohydrate and protein metabolism, would commend the homofermentative lactobacteriaceae as model systems for the basic exploration of protein synthesis. The experimental material to be reported includes data on the amino acids mentioned, the analytical technics employed and their accuracy, as well as data on the relation between growth rates and amino acid assimilation. (Work done under a research contract with the Office of Naval Research.)

Dissolution of fibrin by neutral inorganic salts. SIDNEY SHULMAN AND SIDNEY KATZ (introduced by JOHN D. FERRY). *Dept. of Chemistry, Univ. of Wisconsin, Madison.*

Fibrin, prepared from bovine fibrinogen (at 5 gm/l., pH 6.2, ionic strength 0.45) and thrombin (1 U/ml.) in the absence of calcium, can be dissolved by the following reagents, at the concentrations indicated: lithium chloride (3 M), lithium bromide (2 M), sodium bromide (2 M), and sodium iodide (1 M). Sodium chloride is ineffective, even at a concentration of 4 M. Similar clots can be dissolved by 3 M urea, a somewhat lower concentration than previously employed for this purpose. Clots formed in the presence of 0.001 M calcium chloride, however, fail to dissolve in any of these reagents. (From the insolubility in urea, it is inferred that our fibrinogen preparations contain the serum factor of Laki and Lóránd). When any of the fibrin solutions is dialyzed against 0.45 ionic strength buffer, a gel very similar to the original clot is formed. Ultracentrifuge studies on the dissolved fibrin in each of the salts, as well as on fibrinogen samples to which each salt had been added and subsequently removed by dialysis, showed a single sharp sedimentation peak. This, together with the lack of any turbidity or coagulation upon removal of the concentrated salt, indicates that no appreciable denaturation occurs during the length of time required for fibrin dissolution.

A specific (3-) nucleotidase from barley. LOUIS SHUSTER* AND NATHAN O. KAPLAN. *McCollum-Pratt Inst., Johns Hopkins Univ., Baltimore, Md.*

Germinating barley has been found to contain 3 nucleotidases: a nonspecific acid phosphatase, a specific 5- and a 3-nucleotidase. (We have termed this latter enzyme '3' nucleotidase, as it splits adenylic acid 'b' which is thought to be 3 adenylic acid.) The 3-nucleotidase has been separated by

ammonium sulfate fractionation and further purified by heating, alumina adsorption and alcohol fractionation. The resulting preparation contains only traces of acid phosphatases, ATPase, and 5 phosphatase activity. The enzyme splits the 'b' isomers of adenylic acid and inosinic acid, and commercial guanylic acid. It does not hydrolyze the 'a' and 5 isomers of adenylic acid and inosinic acid, neither does it split the common sugar hexose phosphates, ribose-5-phosphate, phosphoglyceric acid, glycerol phosphate, DPN, or TPN. It will attack commercial uridylic and cytidylic acids, but at respective rates of one third and one fifteenth of that for 'b' adenylic acid. When cytidylic acid is deaminated with nitrous acid, it is split at a rate similar to that of the uridylic acid. The enzyme has an optimum pH of 7.5 (tris buffer), but is active over a wide range. It is quite heat-stable, being only 30% inactivated by heating for 20 minutes at 100°C. Fluoride does not inhibit in concentrations up to 0.1 M. No magnesium activation has been found and there is little loss of activity on dialysis. Equimolar concentrations of 'a' and 5 adenylic have been found to competitively inhibit the splitting of 'b' adenylic by about 15%.

Photosynthetic conversion of acetone to acetate by *Rhodopseudomonas gelatinosa*. JACK M. SIEGEL (introduced by PAUL L. DAY). *Dept. of Biochemistry, School of Medicine, and Inst. of Science and Technology, Univ. of Arkansas, Little Rock.*

Preliminary observations indicated that acetone condenses with CO_2 to form acetoacetate during anaerobic photosynthesis by *R. gelatinosa*, rather than cleaves into C_2 plus C_1 fragments as reported for other biological materials. Acetate has been found to accumulate during the photometabolism of acetone at light intensities that are rate limiting. The ratio of acetate to acetone is inversely related to light intensity and values approaching 2 have been observed under dim light. That this reaction does not take place in complete darkness was shown by the following experiment. Resting cells were incubated with acetone-1-C^{14} and the suspension divided into 3 fractions: Steam volatile acids, insoluble cell material and carbonate. No C^{14} appeared in any fraction. Exposure of a duplicate suspension to dim light gave the following results: 8 μ moles acetone consumed, 15 μ moles acetate produced, molar specific activity of acetate was $\frac{1}{2}$ that of the acetone. Incubation of resting cells with ordinary acetone plus $C^{14}O_2$ resulted in the formation of 18 μ moles of acetate from 16 μ moles of acetone. The molar specific activity of the acetate was approximately half that of the $C^{14}O_2$. Under similar conditions incubation of acetate plus $C^{14}O_2$ resulted in negligible incorporation of the C^{14} into acetate. These results substantiate that acetoacetate and acetate are intermediates in the photosynthetic metabolism of acetone.

Effect of protein depletion on the synthesis of phospholipids, nucleic acids and phosphoprotein in particulate matter of rat liver. S. A. SINGAL, S. J. HAZAN,* V. P. SYDENSTRICKER* AND JULIA M. LITTLEJOHN.* *Depts. of Biochemistry and Medicine, Med. College of Georgia, Augusta.*

Rats fed a protein-free diet for 40–50 days showed a decrease in liver nitrogen, which was reflected in the mitochondrial, microsomatic and residual cytoplasmic fractions. Nuclear nitrogen was not significantly altered. The desoxyribosenucleic and pentosenucleic acid phosphorus did not change in the deficient state. In spite of adequate choline intake the deficient animals exhibited fatty livers, which were not present in animals on 24% casein rations. Studies involving the incorporation of radiophosphorus indicated an increase in relative specific activity of the pentosenucleic acid and phospholipids in the cytoplasmic components of the deficient animals, being most marked in the microsomatic fraction. Phosphoprotein fractions were less affected. Rate studies indicated that at 8 and 12 hours following the administration of radiophosphorus the increase in relative specific activity was significant. Rats on protein repletion experiments showed by the 3rd day a return to normal of particulate matter nitrogen and a drop in relative specific activity of pentosenucleic acid and phospholipids to that observed in animals on adequate protein rations.

Products of the action of pancreatic desoxyribonuclease. ROBERT L. SINSHEIMER (introduced by FRITZ SCHLENK). *Dept. of Physics, Iowa State College, Ames.*

The products of the action of crystalline pancreatic desoxyribonuclease upon highly polymerized calf thymus desoxyribonucleic acid include small amounts of mononucleotides and more appreciable quantities of di-, tri- and higher polynucleotides. By appropriate anion exchange fractionation procedures, involving the use of varying conditions of elution, it has been possible to isolate several di- and tri- desoxyribonucleotides in apparently homogeneous form. Sequentially isomeric dinucleotides have been resolved. These fractions have been identified and characterized by further degradative procedures involving the use of alkaline phosphatase and purified venom phosphodiesterase. The significance of the pattern of these products will be discussed and inferences drawn as to the structure of desoxyribonucleic acid and the mode of action of pancreatic desoxyribonuclease.

Fluorescence and absorption spectra of steroidal estrogens heated in sulfuric acid. WILSON R. SLAUNWHITE, JR.,* LEWIS L. ENGEL, JESSE F. SCOTT* AND CLAIRE HAM.* *Medical Labs. of Collis P. Huntington Memorial Hosp. at Massachusetts General Hosp., Boston; Dept. of Biological Chemistry, Harvard Med. School, Boston and Dept. of Biology, Massachusetts Inst. of Technology, Cambridge.*

The accurate fluorometric estimation of steroidal estrogens depends upon a knowledge of both the fluorescence spectra and the absorption of light by the fluorophor. Therefore, several such compounds were heated in sulfuric acid and the fluorescence spectra measured, using a monochromator to isolate the exciting radiation and a spectrograph to record the emitted radiation. Estrone, both estradiols and estriol had their fluorescence maxima between 480 and 487 mμ; equilin and equilenin at 560 mμ. 6-Ketoestrone, 17-desoxoestrone and 3-methyl-$\Delta^{1,3,5}$-estratriene-4, 17-diol did not fluoresce strongly in the region 440–600 mμ. A mixture of estrone, estradiol-17 β and estriol also had a maximum at 485 mμ, but more of the energy was radiated on the long wavelength side than in the case of the separated components of the mixture. A phenolic fraction from normal male urine had a low broad fluorescence spectrum after heating with sulfuric acid. A similarly treated phenolic fraction from the urine of a male patient who had received estriol had a typical estrogen fluorescence spectrum superimposed upon that of the normal male urine extract. The fluorescence of estrogens measured at 488 mμ and at 525 mμ was found to be additive when either pure estrogens or estrogens and phenolic urine extracts were used. All of the estrogens tested absorbed strongly between 360 and 530 mμ after heating in sulfuric acid under similar conditions.

Factors affecting the oxidation of aniline by *Mycobacterium smegmatis*. N. H. SLOANE, M. SAMUELS, C. RITTER, C. CRANE AND R. L. MAYER (introduced by J. J. OLESON). *Lederle Labs. Div., American Cyanamid Co., Pearl River, N. Y., and Ciba Pharmaceutical Products Co., Summit, N. J.*

Sloane, Crane and Mayer (*J. Biol. Chem.* In press) have described that *M. smegmatis* oxidizes aniline to *p*-aminophenol. Further studies on this problem were directed towards determining the effect of various factors on this oxidation. There is no sharply defined pH optimum, the reaction proceeds equally well from pH 5.0–7.4. The addition of manganese to the otherwise complete system greatly stimulates the oxidation of aniline. Magnesium or ferrous ions do not replace manganese in the reaction. Phosphate is necessary for this oxidation. The oxidation of aniline is inhibited by arsenate, dinitrophenol, fluoride, arsenite, cyanide, azide and iodoacetate (5–10 hour runs). Arsenate and dinitrophenol do not markedly affect the uptake of oxygen by these cells, but these compounds do inhibit the aniline oxidation. The following facts indicate that the oxidation of aniline is an energy coupled reaction: *a*) the necessity of phosphate, *b*) the inhibition of the reaction by compounds which inhibit oxygen uptake and *c*) the inhibition of the reaction by compounds which uncouple phosphorylation from oxidation.

Identification of glycyl-alanyl-glycine in silk hydrolysates. E. SLOBODIAN* AND M. LEVY. *Dept. of Chemistry, New York Univ. College of Medicine, New York City.*

Several theories of silk fibroin structure suggest the sequence, -glycine-alanine-glycine-, as an important unit, but the corresponding tripeptide has not been isolated. We have now been able to demonstrate the presence of significant amounts of glycyl-alanyl-glycine in partial hydrolysates of fibroin. Analyses for this tripeptide were done by the isotopic derivative 'carrier' method, as previously applied to dipeptides (M. LEVY AND E. SLOBODIANSKY *Federation Proc.* 7: 168, 1948). Known amounts of synthetic nonisotopic pipsyl (*p*-iodo-phenyl-sulfonyl) glycyl-alanyl-glycine 'carrier' were added to partial hydrolysates which had been treated with radioactive (I^{131}) pipsyl chloride. The carrier was purified to constant specific activity, and from the latter value, the amount of glycyl-alanyl-glycine in each hydrolysate was calculated. At this stage, the carrier was chromatographically pure. Control mixtures gave zero analyses in the absence of glycyl-alanyl-glycine, and about 80% recovery when this peptide was present. The amounts of glycyl-alanyl-glycine found in 8-hour and 24-hour silk hydrolysates were in excess of 5.1% of total nitrogen, the maximum value to be expected in a random structure. These findings support the view of an ordered amino acid arrangement in fibroin, with glycine-alanine-glycine- as an important structural element.

Effect of cold on arterial reactivity in vitro. DURWOOD J. SMITH (introduced by WILLIAM S. MCCANN). *Dept. of Medicine and Physiology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Variations in arterial reactivity caused by hypothermia have been studied in 133 isolated surviving dog, swine and human arteries employing an angioplethysmokymographic technique (*Circulation* 4: 890, 1951). Vasoconstriction by cold is a function of the rate of cooling. Arteries cooled at 4°/minute constrict about 34 ± 5.5%

of their volume, while cooling at 2.5°C. or below/-minute produces insignificant constriction. In 23 experiments, flow through the vessel wall (vasa vasorum) at 37°C. averaged 9.7 ± 1.77 cu. mm/10 minutes; 4 ± .45 cu. mm/10 minutes at 27°C. and 1.45 ± .41 cu. mm/10 minutes at 17°C. The vasoconstriction caused by epinephrine, acetylcholine and histamine is prolonged 450–700% at 17°C. over control values obtained at 37°C. The prolongation of the reaction time of arteries at low temperatures is due to a lengthened 'constriction time' as well as a delay in the recovery phase of the vascular smooth muscle. These results will be discussed in relation to the physiology of cold injury.

Bacterial cytochromes. LUCILE SMITH (introduced by BRITTON CHANCE). *Johnson Research Fndn., Univ. of Pennsylvania, Philadelphia.*

A quantitative study of the cytochromes of bacteria was made, using the method devised by Chance (*Federation Proc.* 10: 171, 1951) for measuring the difference spectra of pigments in whole cells. The proper suspending media were found in order to eliminate nonspecific changes in optical density. Complete spectra in the visible and Soret regions for the difference between the oxidized and reduced states of the cytochromes were obtained for a number of kinds of bacteria. The absorption peaks observed are as follows:

	VISIBLE	SORET
Escherichia coli.....	627, 591, 560, 532	432, shoulder at about 437
Aerobacter aerogenes.....	627, 591, 560, 530	430 shoulder at about 436
Azotobacter chroococcum...	552, 520	428
Acetobacter pasteurianum..	556, 528	428, shoulder at about 440–445

The positions of the visible bands agree with previous visual observations, but we have now a quantitative measure of the relative amounts of the cytochromes present. The Soret bands of the bacterial cytochromes are roughly symmetrical about 430 mμ and are dominated by cytochrome *b* (or b_1); other pigments only appear as slight shoulders on the Soret band. The pigments of these bacteria are only slightly more difficult to measure than those of a heart muscle homogenate, as shown by values for the ratio of the Soret peak to the total turbidity; representative figures for bacteria range from 5–30, as compared with values of about 80–100 for heart muscle particles. Values for oxidase activity expressed in terms of the absorption in the Soret region fall within a surprisingly small range, in view of the considerable differences in Q_{O_2} values. Rates of oxygen uptake divided by the increases in optical density at the Soret peak on reduction give values of 43–126 for the bacteria; similar values for heart muscle extract and yeast are around 80 and 220, respectively.

Metabolic relation between methionine and adeninethiomethylriboside (thiomethyladenosine). RAYMOND L. SMITH* AND F. SCHLENK. *Dept. of Bacteriology, Iowa State College, Ames.*

Saccharomyces cerevisiae and *Torulopsis utilis* contain less than μM of 5′-thiomethyladenosine/gm. dry matter, if grown under ordinary conditions. Fortification of the medium with methionine leads to accumulation of the nucleoside in yeast. With an excess of the amino acid about 20 μg/moles (0.6%) of thiomethyladenosine/gm. yeast may be obtained. The response to methionine is specific; sulfate, homocystine, cysteine, and glutathione do not show any comparable effect. On transfer of yeast which is rich in thiomethyladenosine into a culture medium devoid of sulfur supply but otherwise adequate, the nucleoside is metabolized. This suggests a function as sulfur reserve. A new method of preparation of adeninethiomethylriboside and new analytical techniques have been developed. The nucleoside can be determined by the nitroprusside reaction in the same way as methionine. Phosphotungstic acid serves for separation of the 2 compounds. The mode of the reaction and the size of the fragment which methionine contributes toward formation of the nucleoside will be discussed.

Insulin resistance produced by insulin-hypoglycemia. MICHAEL SOMOGYI. *Lab. of the Jewish Hosp. of St. Louis, St. Louis, Mo.*

Insulin, when allowed to produce hypoglycemia, defeats its own action by mobilizing insulin-antagonistic hormones. We demonstrated this fact by 4 different experimental approaches (*J. Biol. Chem.* 174: 597, 1948; *J. Biol. Chem.* 179: 217, 1949; *Endocrinology* 47: 436, 1950; *J. Biol. Chem.* December, 1951). The present study was based on the postulate that since the insulin antagonists are products of the adrenal-pituitary system, excitation by repeated hypoglycemic states may enhance the capacity of these glands to produce and secrete blood sugar-raising hormones beyond the normal limits. Such response should increase the ability of animals to combat the hypoglycemic action of insulin and should also be reflected in anatomic and cellular changes in the glands. Rabbits were given repeated insulin injections, 1 U/kg/day in a single dose, inserting 1 or several insulinless days for recovery. It was observed that in the course of a few weeks of such treatment both the intensity and duration of insulin action gradually diminished. The dosage was then gradually increased, with the consequence that 3, and even 4 U/kg. could be injected without producing any visible effect; in fact, 3 units produced less hypoglycemia than did 1 unit at the outset. The anatomic changes in the endocrine

organs also materialized as anticipated; hyperplasia and cellular changes, indicating increased activity, were in evidence. Thus it may be stated that *insulin-hypoglycemia engenders insulin resistance by developing the capacity of the adrenal-pituitary system to produce and secrete insulin-antagonistic hormones above the normal levels.*

Nitrogen precursors of hypoxanthine. JOHN C. SONNE AND I. LIN (introduced by JOHN M. BUCHANAN). *Dept. of Physiological Chemistry, School of Medicine, Univ. of Pennsylvania, Philadelphia.*

N^{15}-labeled substrates have been added to pigeon liver extracts synthesizing hypoxanthine from radioactive carboxyl-labeled glycine. The specific activities of the labeled compounds were determined at the beginning and at the conclusion of a 30-minute incubation and were found to remain relatively constant. From the N^{15} and C^{14} content of the isolated hypoxanthine could be calculated the number of molecules of N^{15}-substrate incorporated into each molecule of hypoxanthine newly synthesized from carboxyl-labeled glycine. It is known that nitrogen atom number 7 of the purine ring is derived from glycine. Three molecules of some other nitrogen source have been regarded as donating the other 3 purine ring nitrogens. In pigeon liver extracts only one quarter of 1 molecule of ammonia is incorporated into each molecule of newly synthesized hypoxanthine. Hence ammonia cannot be regarded as a direct precursor of any 1 of the purine nitrogen atoms. Somewhat less than 1 molecule of N^{15}-aspartic acid is incorporated into 1 molecule of hypoxanthine synthesized from carboxyl-labeled glycine. Hypoxanthine has not been degraded to determine whether this N^{15} resides solely in 1 of the 3 nitrogens not donated by glycine, or is distributed generally among them. Glutamic acid and glutamine are being studied in a similar manner as possible precursors.

Substrate specificity of bacterial tyrosine decarboxylase and kidney DOPA decarboxylase. THEODORE L. SOURKES AND PETER HENEAGE (introduced by WAYNE W. UMBREIT). *Merck Inst. for Therapeutic Research, Rahway, N. J.*

Enzymes obtained from *S. fecalis* and from pig and guinea pig kidney were used in a study of the comparative rates of decarboxylation of isomers of 3,4-dihydroxyphenylalanine (3,4-DOPA) and some of its derivatives. The relative rates for the isomers examined were as follows:

	ENZYME BACTERIAL	KIDNEY
3,4-DOPA	100	100
2,5-DOPA	15	88
3,5-DOPA	27	24
2,4-DOPA	115	71

N-methyl- and N-(2-cyanoethyl)-3,4-DOPA are not decarboxylated by the kidney enzyme. 3,4 dihydroxyphenylserine was not appreciably decarboxylated by kidney enzymes in our experiments, but was acted upon by preparations of beef adrenal medulla. Data will also be presented on the decarboxylation of methoxylated derivatives of DOPA. In general, the rules of substrate specificity of DOPA decarboxylase, elaborated by Blaschko, have been confirmed.

Effects of ultraviolet irradiation upon nucleic acids and sodium nucleates. MONA SPIEGEL-ADOLF. *Dept. of Colloid Chemistry, Temple Univ. School of Medicine, Philadelphia, Pa.*

Discrepancies exist in the literature (HEYROTH AND LOUFBORROW. *J. Am. Chem. Soc.* 53: 3441, 1931; GREENSTEIN AND JENRETTE, *Cold Spring Harbor Symp.* 9: 236, 1941) about changes produced in the ultraspectrophotogram of nucleic acids by ultraviolet radiation. A commercial preparation of sodium desoxyribose nucleate was used which spectrophotometrically checked satisfactorily with an original Hammarsten preparation. A Hanovia mercury quartz lamp, d.c. 220 v. was used as light source. The distance of the object to the burner was 20 inches which made cooling devices unnecessary. The ultraspectrographic measurements were made with a Beckman quartz photoelectric spectrophotometer. In view of the shading effect noticed in relatively high concentrations of water soluble chlorophyll (*Federation Proc.* 6: 294, 1947) a series of irradiations were made in which the concentration of the nucleate varied from 0.5–0.001%. While the higher concentration showed only 6% decrease in optical absorption after 12 hours irradiation the lowest concentration retained only one third of the original selective absorption power at 260°mμ If a quartz cell 5 mm. thick is filled with 0.5% nucleic acid and put in the light beam in front of a quartz test tube containing 0.001% sodium nucleate, the latter after a 4-hour irradiation remains unchanged. On the other hand light of 365 mμ wave length (Wood filter) of a comparable intensity is practically without effect upon the optical absorption of sodium nucleates. Irradiation in a nitrogen atmosphere of sodium nucleates and their constituent purines is apparently much less effective than irradiation in air.

Effects of ultrasonic radiation upon nucleic acids and sodium nucleates. M. SPIEGEL-ADOLF AND P. J. ERNST.* *Temple Univ. School of Medicine, Dept. of Colloid Chemistry and College of Liberal Arts, Dept. of Physics, Philadelphia, Pa.*

Ultrasonic radiations have been used recently in diagnosis and therapy. Studies of possible effects of such radiations upon cell constituents seem therefore warranted. Until now only sonic

radiations (up to 9000 cycles) have been used on nucleic acids (KREJCI, SWEENEY AND HAMBLETON. *J. Franklin Inst.* 248: 177, 1949; GOLDSTEIN AND STERN, *J. Polymer. Sc.* 5: 687, 1950). An apparatus of the Piezo Product Company was available, an output of 100 ma. was used, the frequencies varied between 500–5000 kilocycles. The solutions were irradiated for 15–60 minutes in closed plastic vessels, the radiations were concentrated with the help of lucite lenses. Cavitation was observed in every case. The temperature varied between 25–57°. For all temperatures above 37° special controls were made. The concentration of the solutions varied between 0.01 and 0.5%. Commercial samples of both desoxyribose and ribose nucleic acids were used which conformed in their optical absorption to a standard sample. The optical absorption over a range of 310–220 mμ was measured with the help of a Beckman Quartz photoelectric spectrophotometer. In 12 various experiments the selective absorption power remained practically unchanged after the exposure of the samples to ultrasonic radiation. Results obtained with the help of a Zeiss interferometer do not permit to assume any marked degree of polymerization. These findings seem to preclude nucleic acids as points of attack of ultrasonic radiation in tissues within the studied wave lengths. (See HERRICK *et al. A.M.A. Exhibit*, 1951.)

Observations with isotopically labeled insulin. WILLIAM C. STADIE AND NIELS HAUGAARD.* *Univ. of Pennsylvania, Philadelphia.*

Insulin was sulfated in the cold using $H_2S^{35}O_4$. The preparations contained 2–3% of sulfate sulfur and 3–4 million cpm/mg. Approximately full biological activity was retained. The isotopic preparation made it possible to measure insulin in minute quantities. In control experiments approximately 100% of insulin added to tissues could be quantitated down to about 0.01 μg. Distribution experiments after intravascular injection into rats of isotopic insulin were done. In addition, insulin bound by diaphragm and other tissues was measured under a variety of conditions. Accurate measurements of the relation between bound insulin and its biological effects were made. A variety of other experiments bearing on the problem of the binding of insulin by tissues are also reported.

Acyl-coenzyme A synthesis by phosphotransacetylase and coenzyme A-transphorase. E. R. STADTMAN. *Section on Cellular Physiology, Natl. Insts. of Health, Bethesda, Maryland*

Extracts of *C. kluyveri* catalyze the over-all reaction (*1*) Acetyl-P + Propionate ⇌ Propionyl-P + Acetate. Recent studies indicate that this reaction is the net result of the following coupled reactions: (*2*) Acetyl-P + CoA ⇌ Acetyl-CoA + Pi; (*3*) Acetyl-CoA + Propionate ⇌ Propionyl-CoA + Acetate; (*4*) Propionyl-CoA + Pi ⇌ Propionyl-P + CoA. *Reaction 1* does not occur in the absence of CoA. Experiments with P^{32}-labeled acetyl-P carried out in the presence of a large excess of unlabeled orthophosphate, show that the phosphoryl group of the propionyl-P (*reaction 1*) is derived from orthophosphate. A direct phosphoryl group transfer from acetyl-P to propionate is excluded as a major pathway. Proof for *reaction 2* was obtained by incubating purified phosphotransacetylase with acetyl-P and CoA. Acetyl-CoA which accumulated in almost quantitative yield was isolated by paper chromatography and was identified as an acetyl mercaptan derivative of CoA (cf. LYNEN *et al. Annalen der Chemie* 574: 1, 1951). It is enzymatically active in the synthesis of citrate by Stern and Ochoa's crystalline condensing enzyme. *Reaction 3* was demonstrated by incubating acetyl-CoA and propionate with dialyzed extracts of *C. kluyveri* in the almost complete absence of inorganic phosphate. The formation of propionyl-CoA (60% yield) was estimated by paper chromatography of the hydroxamic acid derivatives. *Reaction 4* is probably also catalyzed by phosphotransacetylase since this enzyme catatlyzes the arsenolysis of propionyl-P. The enzyme catalyzing *reaction 3* has been called coenzyme A-transphorase (BARKER. *Phosphorus Metabolism*, 1951).

Microbiological degradation of cholesterol. THRESSA C. STADTMAN AND AMELIA CHERKES (introduced by CHRISTIAN B. ANFINSEN). *Section on Cellular Physiology, Natl. Insts. of Health, Bethesda, Maryland.*

The degradation of cholesterol has been studied in a saprophytic Mycobacterium capable of growth on the sterol as a sole carbon source. C^{14}-labeled cholesterol, prepared biosynthetically from 1-C^{14}-acetate, was degraded by growing cultures completely to CO_2 as estimated both by radioactive and chemical determinations. Glycerol-grown cells in contrast to those grown on cholesterol produce cholestenone as the only product. Oxidation of cholesterol beyond this stage thus appears to involve an adaptive enzyme system. The enzyme carrying out the conversion of cholesterol to cholestenone has been partially purified from extracts of both glycerol and cholesterol-grown cells. The ability to form CO_2 from cholesterol is lost when cell-free extracts are prepared from sterol-grown cells. However, these extracts convert cholesterol to a variety of products which have been partially resolved by silica gel and alumina chomatography. These fractions have been subjected to infrared and ultraviolet spectrophotometric analysis. Compounds showing in

common a functional group that absorbs in the infrared at 1735 cm.$^{-1}$ appear in the fractions eluted from columns by solvent systems of varying degrees of polarity. Other fractions, also differing in polarity, contain compounds possessing absorption bands at 1667 cm.$^{-1}$ characteristic of α - β unsaturated ketones. Reactivity with carbonyl reagents has been determined and various keto steroids have been separated as their 2,4 dinitrophenyl hydrazones by means of paper chromatography. Radioactivity appears in the keto steroids formed from 4-C^{14}-cholestenone by cell-free extracts.

Isolation studies with bovine thyrotropin. S. L. STEELMAN, J. W. GIFFEE, JR. AND E. J. HAWRYLEWICZ (introduced by EDWIN E. HAYS). *Fundamental Research Dept., Armour Labs., Chicago, Ill.*

A number of methods have been employed to isolate purified bovine thryotropin. Using fractional electrical trnasport preparations containing approximately 10 U. S. P. U/mg. (as measured by the iodine depletion from the thyroids of day-old white leghorn cockerels) have been obtained. Total doses of approximately 2 μg. deplete 50% of the iodine from chick thyroids in 3 days without significant change in weight. Somewhat larger doses administered over a period of 5 days give both iodine depletion and gland weight responses. Gonadotropic activity at these and higher dosage levels is absent. As evidenced by the above method and paper electrophoresis the isoelectric point of bovine thyrotropin is between 8.0 and 8.5. Preliminary examination indicates the preparations are nearly homogeneous. Using starting materials made by different methods and having varying potencies, the thyrotropic activity always concentrates at pH's above 7.0 indicating there are no fractions having appreciable activity with lower isoelectric points. Amino acid analysis by means of paper chromatography shows the presence of large amounts of both acidic and basic amino acids. Methionine and phenylalanine are apparently absent. All preparations have small amounts of carbohydrate, give good ninhydrin colors and have ultraviolet absorbing qualities.

Evidence for intermediate compounds in the biosynthesis of ovalbumin. D. STEINBERG,* AND C. B. ANFINSEN. *Section on Cellular Physiology, Natl. Insts. of Health, Bethesda, Md.*

It has been reported previously that when ovalbumin is synthesized *in vitro* by hen's oviduct in the presence of $C^{14}O_2$, the aspartic acid residues at different positions in the molecule do not all have the same specific activity, i.e. the ovalbumin molecule is nonuniformly labeled (*J. Biol. Chem.* 189: 739, 1951). Nonuniform labeling has now been demonstrated for several other amino acids split from ovalbumin with the *B. subtilis* enzyme of Linderstrøm-Lang: glutamic acid, alanine and glycine. In *in vitro* incubations, as much as one third of the $C^{14}O_2$ fixed in the ovalbumin aspartic acid was located in the alpha carboxyl group. Nonuniform labeling of ovalbumin has been shown to hold for both the alpha (peptide bound) and beta carboxyl groups of aspartic acid. C^{14}-alanine labeled ovalbumin was partially degraded with pepsin and the resulting mixture of peptides was separated into 30–40 arbitrary fractions by paper chromatography using 4 different solvent mixtures. The specific activities of the alanine samples isolated from these fractions were determined by a micro method involving direct counting of 5–20 γ in a flow counter followed by direct colorimetric analysis. The values obtained ranged from 1.0–5.5 c/m/γ. Labeled ovalbumin was prepared *in vivo* by intraperitoneal injection of $NaHC^{14}O_3$ into hens. Nonuniform labeling with respect to aspartic acid was demonstrated, confirming the *in vitro* results. Nonuniform labeling does not appear to be compatible with theories proposing a simultaneous assembling of free amino acids onto a master template. The findings require the postulation of intermediate compounds in ovalbumin synthesis, probably peptide in nature. As one possible intermediate, glycine-labeled glutathione was synthesized to compare the efficiency of glutathione as a glycine donor in protein synthesis with that of glycine itself.

Utilization of methionine-CH_3-C^{14}, glycine-2-C^{14}, serine-3-C^{14}, and formate-C^{14} for the synthesis of choline and creatine by chicks, weanling rats, and mice. JAKOB A. STEKOL, SIDNEY WEISS,* BETTY HSU* AND PHYLLIS SMITH.* *Lankenau Hosp. Research Inst. and Inst. for Cancer Research, Philadelphia, Pa.*

Weanling rats and 2-week-old pullets maintained on a diet consisting of a mixture of amino acids free of methionine, cystine, glycine, serine, and choline, but containing homocystine and all the known vitamins, utilized methionine-CH_3-C^{14}, glycine-2-C^{14}, serine-3-C^{14}, or formate-C^{14} for the synthesis of choline and creatine. The extent of the synthesis of choline from these precursors in the chick will be compared with that obtained on weanling rats, and the effect of various additional dietary components on the extent of choline formation in both species will be illustrated. In adult mice, as in adult rats (STEKOL, WEISS AND WEISS *Federation Proc.* 10: 252, 1951; *Am. Chem. Soc. 120th Meeting, 1951, New York*, abstracts 21-C), the utilization of glycine-2-C^{14}, but not of serine-3-C^{14}, for choline synthesis is reduced in vitamin B_{12} deficiency. Folic acid deficient mice, like rats, show a reduced capacity to utilize either glycine-2-C^{14} or serine-3-C^{14} for choline formation. The

administration of Citrovorum Factor (Leucovorin, Lederle) intraperitoneally or of folic acid with food to folic-acid-deficient mice augmented the utilization of glycine-2-C^{14} and serine-3-C^{14} for choline formation. (The work was supported in part by grants from the National Cancer Institute, U.S. Public Health Service, and from Borden Company, and the radioactive material was obtained through the U.S. Atomic Energy Commission.)

Mechanism of enzymatic synthesis of citric acid. JOSEPH R. STERN,* SEVERO OCHOA, AND FEODOR LYNEN.* *Dept. of Pharmacology, New York Univ. College of Medicine, New York City, and Chemical Inst., The University, Munich, Germany.*

The isolation and elucidation of the nature of acetyl CoA (LYNEN, F., AND E. REICHERT, *Angewandte Chem.* 63: 47, 1951) has made it possible to obtain final proof that crystalline condensing enzyme (J. R. STERN *et al. J. Biol. Chem.* 193: 703, 1951) catalyzes the following reaction:

$$CH_3{-}CO{-}SR + {}^-OOC{-}CO{-}CH_2{-}COO^- +$$

$$H_2O \rightleftharpoons {}^-OOC{-}CH_2{-}\underset{\displaystyle COO^-}{\overset{\displaystyle OH}{\overset{|}{\underset{|}{C}}}}{-}CH_2{-}COO^- + HSR + H^+$$

where $CH_3{-}CO{-}SR$ and HSR represent acetyl CoA and CoA, respectively. Synthesis of citric acid is accompanied by liberation of a stoichiometric amount of SH-groups. The equilibrium constant of the reaction has been measured spectrophotometrically through coupling with the malic dehydrogenase system. The over-all reaction (acetyl CoA + malate = + $DPN^+ + H_2O \rightleftharpoons$ citrate ≡ + CoA + DPNH + $2H^+$) is strongly pH-dependent. From the value of the equilibrium constants of the above reaction and that catalyzed by malic dehydrogenase at pH 7.2, it can be calculated that the free energy of the acyl-mercaptide bond in acetyl CoA is of the order of 12,000 gm. cal/mole. This agrees well with the value calculated using the equilibrium constant of the reaction catalyzed by transacetylase (acetyl phosphate + CoA ⇌ acetyl CoA + phosphate) recently determined by Stadtman (Gordon Research Conferences, 1951).

Nature of biochemical mutants of *Neurospora crassa* produced by sulfur mustards. CARL M. STEVENS AND AUGUSTA MYLROIE.* *Fulmer Chemical Lab., State College of Washington, Pullman.*

In attacking the problem of the mechanism of mutagenic action of mustard gas and related compounds, we were interested in extending previous studies (*Federation Proc.* 10: 253, 1951) of the monofunctional alkyl 2-chloroethyl sulfides by determining whether treatment of wild-type *Neurospora crassa* with these compounds would produce biochemical mutants. Horowitz *et al.* (*Science* 104: 233, 1946) have demonstrated that mustard gas itself is effective. If such mutants could be produced, it would be of interest to test the effect of a second treatment with a sulfur mustard, or other mutagen, in inducing a change back to nutritional independence, and then to test genetically whether such changes were true back-mutations. Treatment of macroconidia of *Neurospora crassa* (strain 1a newly reisolated from a cross of Em5256A and Em5297a) with aqueous acetone solutions of *n*-butyl 2-chloroethyl sulfide (0.002–0.01M) produced an array of biochemical mutants, which were isolated according to the procedure of Lein *et al.* (*Proc. Natl. Acad. Sc.* 34: 435, 1948). One experiment yielded mutants with the following nutritional requirements: cytidine 4, inositol 2, leucine 1, choline 1, cystine 1, arginine 1, threonine or isoleucine 1. In 3 comparable experiments using mustard gas, mutants isolated possessed these requirements: methionine 7, lysine 5, PABA 4, adenine 3, leucine 2, cytidine 2, inositol 2, niacin 1, serine 1, proline 1, arginine 1. Using one of the above PABA-requiring strains, experiments of the type employed in earlier studies (*loc. cit.*) have shown that treatment with mustard gas, *n*-butyl 2-chloroethyl sulfide, or ultraviolet light is effective in producing PABA-independent forms. Genetic tests are being applied to establish whether the development of nutritional independence is the result of genetic back-mutation.

4-Amino-5-imidazolecarboxamide. Absence of correlation between its accumulation and the inhibition of bacterial growth by sulfonamides. RICHARD C. STEWART* AND M. G. SEVAG. *Dept of Microbiology, School of Medicine, Univ. of Pennsylvania, Philadelphia.*

It has been postulated that sulfonamides through the agency of a 'folic acid' block the the incorporation of 'formate' into position 2 of the purine molecule leading to the accumulation of 4-amino-5-imidazolecarboxamide or a derivative of this amine, thus contributing to the inhibition of growth. Our findings are: *1*) A nonacetylable, diazotizable amine does not accumulate in casein hydrolysate medium free from glucose. The maximal amount we have obtained during a sulfonamide-inhibition of growth of 59% is about 0.56 μg/ml. of 0.7 μg/100 μg. *E. coli* (Strain B). *2*) A similar measurable amount, 0.26 μg.–1.58 μg/ml. accumulates also during growth in medium containing glucose but free from sulfonamide. *3*) In the presence of glucose and sulfonamide, the

amount is increased up to 13.4 μg/100 μg. cells or 19.24 μg/ml. during an inhibition of growth of 63%. This increment is obviated when the system is magnetically stirred (aerated). This stirring does not destroy the accumulated amine. Accumulation of the amine is therefore accelerated 20-fold by glucose metabolism in the presence of sulfonamide. Rechanneling of this metabolism by magnetic stirring (aeration) blocks the accumulation of this amine without, however, affecting the degree of growth inhibition. Furthermore, there lacks a constant relationship between the amount of the amine formed and degrees of growth inhibition. These findings show that the inhibition of growth by sulfonamides and the accumulation of the amine as purine intermediary are not interdependent.

Spectrophotometric estimation of fecal estrogens in pregnant women. BENJAMIN F. STIMMEL. *Rees-Stealy Clinic Research Fndn., San Diego, Calif.*

Our modified procedure (*Federation Proc.* 9: 235, 1950) for the fractionation and photometric estimation of urinary estrogens has been adapted to human feces. The 24-hour stool is mixed with 50 gm. of Celite filter-aid and 500 ml. of ethyl alcohol and homogenized in the Waring blendor. The homogenate is desiccated under reduced pressure and the finely pulverized residue is extracted at room temperature with ethyl alcohol. The alcoholic extract is mixed with an equal volume of water and washed with petroleum ether. The alcoholic extract is then evaporated almost to dryness and diluted with 200 ml. of sodium borate-boric acid buffer, pH 8.5, and extracted with ethyl ether. The ether extract contains the unconjugated estrogens and the aqueous phase contains the conjugated estrogens. Phenol-like fractions from these extracts are prepared for chromatographic fractionation and spectrophotometric estimation of estrone, estradiol, and estriol. The 24-hour fecal excreta collected during the last trimester of pregnancy yielded by this procedure estrone, estradiol, and estriol residues which produced typical color reactions with the Kober reagent. In a typical case the 24-hour feces contained 20, 46, and 2273 μg., respectively of unconjugated estrone, estradiol, and estriol. In the acid-hydrolyzed aqueous extract, 337 μg. of estriol were present whereas estrone and estradiol were undemonstrable. The 24-hour urine collected simultaneously contained no detectable unconjugated estrogens, whereas conjugated titers following hydrolysis were 190, 170, and 9500 μg., respectively of estrone, estradiol, and estriol. Aliquots of the fecal and urinary estrogenic residues produced characteristic absorption spectra when heated with 90% sulfuric acid.

Isolation of protogen. E. L. R. STOKSTAD AND J. V. PIERCE.* *Lederle Labs. Div., American Cyanamid Co., Pearl River, N. Y.*

Protogen is an unidentified growth factor which was originally shown to be required by the protozoon *Tetrahymena geleii*. Concentrates containing this factor have been found to be essential for the growth of a strain of *Corynebacterium bovis* and to have activity for *Streptococcus faecalis* on an acetate-free medium. Protogen is effective in reversing the inhibitory effect of sodium propionate for *S. faecalis*. Protogen in natural materials such as liver and other animal organ tissue exists largely in a nondialyzable form. Hydrolysis with alkali converts this into a dialyzable state and increases the solubility of protogen in organic solvents. The existence of a number of different biologically active forms of protogen in alkaline hydrolysates of liver can be demonstrated by paper chromatography. Protogen was obtained from liver by counter-current distribution and by chromatographic adsorption. As purification proceeded, the protogen as originally present in the alkaline hydrolysate was converted into another form which was relatively less soluble in organic solvents. These 2 forms are termed, respectively, 'Protogen A' and 'Protogen B.' By paper strip chromatography with wet *n*-butanol in an ammoniacal atmosphere Protogen A has an R_f of 0.70 and Protogen B of 0.33. Protogen B was obtained as a yellow oil and as the crystalline *S*-benzylthiuronium salt. Chemical properties and degradation reactions of Protogen A and Protogen B will be discussed.

Quantitative determination of low concentrations of nitrate by enzymatic reduction. W. R. STRAUGHN (introduced by J. LOGAN IRVIN). *Depts. of Bacteriology and Biochemistry, School of Medicine, Univ. of North Carolina, Chapel Hill.*

The principle involved in this test for nitrates is that of quantitatively reducing nitrate to nitrite by means of bacterial nitratase followed by a photometric determination of nitrite. Cell suspensions of a strain of *Escherichia coli* are used as a source of enzyme and are incubated aerobically with suitable dilutions of the unknown nitrate solution in the presence of sodium lactate to serve as an electron donator. After centrifugation the supernatant is tested for the amount of nitrite present by means of a diazo reaction using sodium para-aminohippurate and N-(1-naphthyl)-ethylenediamine dihydrochloride. Optical densities are determined with a photoelectric photometer. The method is sensitive to 2 γ of nitrate and the response is linear over at least a 10-fold range yielding approximately 90% recovery of nitrate as nitrite. Application of the method for body

fluids is reliable. Details of the procedure and standardization will be discussed.

Firefly luminescence in the study of biological energy transfer systems. B. L. STREHLER AND J. R. TOTTER (introduced by WALDO E. COHN). *Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn., and Biochemistry Dept., Univ. of Arkansas, Little Rock.*

It has been shown previously by McElroy and co-workers that extracts of firefly lanterns will luminesce when a divalent ion, 'luciferin,' oxygen and ATP are present. Theoretically any of these factors might be made rate limiting and assayed over a range of concentration by measuring the light output of the system. Since highly sensitive light measuring apparatus is available, and since the response of the luminescent system is linear over a wide range of ATP concentrations, we have developed methods for assaying minute quantities of ATP, other phosphorylated compounds and certain enzymes. For routine measurements the commercially available Farrand photofluorometer has been used to determine ATP concentrations as low as 1 μg/ml. while a thousandfold increase in sensitivity has been made possible through the use of a more highly refined 'quantum counter.' The following substrates have been assayed by this method: ATP, ADP (pretreatment with myokinase), phosphocreatine (pretreatment with creatine-adenylic transphosphorylase plus adenylic acid), glucose (measuring luminescence in the presence of hexokinase and ATP). Conversely, the enzymes mediating these transformations as well as apyrases have been determined. Changes in ATP concentration in rabbit liver homogenates mixed with firefly enzyme and the proper substrates have been determined as well as some preliminary measurements of the influence of light on ATP levels in illuminated plants. Advantages of the method include sensitivity and accuracy; limitations include instability at low buffer concentrations and inhibition by monovalent anions.

Microchemical determination of several dehydrogenase enzymes in rabbit brain. JACK L. STROMINGER* AND OLIVER H. LOWRY. *Dept. of Pharmacology, Washington Univ., St. Louis, Mo.*

Studies of the rate of reduction of DPN by rabbit brain homogenate in the presence of lactate, malate or glutamate including evaluation of K_M for substrate and DPN, pH optimum and Q_{10}, studies of stability, and purification have been performed. An assay has been worked out in which 1–10 μg. samples of homogenate or frozen dried sections are dispersed in 0.1 ml. of substrate mixture in simply constructed Beckman tubes. Samples, obtained from various regions of rabbit brain, were relatively homogeneous as to component, i.e. cells, dendrites, unmyelinated fibers or myelinated fibers. The method of obtaining these samples and their volumes, to which the rate determinations were related, will be presented. Data have been obtained for malic and lactic activity in each of the layers of Ammon's horn, hypothalamus, supraoptic nucleus, optic tract, and several of the layers of cerebellum and cerebrum. The absolute quantity of each activity varied considerably both among the layers of a region and from region to region. Despite this variation in the quantity of activity, the various layers of the regions studied had similar ratios of malic/lactic activity, but this ratio varied from region to region. The ratio of malic/lactic activity was 0.43 in optic tract, 3.9 in cerebellar white matter, and 2.0 in white matter of Ammon's horn. Other layers of cerebellum and Ammon's horn had ratios corresponding to white matter, and several layers of Area 32 of cerebrum had ratios around 3. (Supported in part by an American Cancer Society Grant.)

Synthesis of higher fatty acids by slices of maturing peanut cotyledons. P. K. STUMPF AND ELDON H. NEWCOMB.* *Div. of Plant Biochemistry, Univ. of California, Berkeley.*

The synthesis of long chain fatty acids by slices of maturing peanut cotyledons has been studied with radioactive substrates. Following 6-hour incubation with substrate, respiratory CO_2 and unused substrate were recovered, the slices were pulped and saponified, and the fatty acids of chain length longer than 10 carbon atoms isolated by the copper-lime procedure. Although pronounced percentage recoveries of the initial radioactivity were obtained in the respiratory CO_2 with all carboxyl-labeled substrates (approx. 31% for formate-C^{14}, acetate-1-C^{14}, and butyrate-1-C^{14}, and 45% for caproate-1-C^{14}), incorporation of C^{14} into the higher fatty acids differed greatly, acetate-1-C^{14} contributing 17%, butyrate-1-C^{14} and caproate-1-C^{14}, 1.8 and 2.7% respectively, and formate 0.3%. However, with acetate-2-C^{14}, less C^{14} appeared in the CO_2 (12%) but more in the fatty acids (24%) than with acetate-1-C^{14}. Only 0.6% of the C^{14} from valerate-3-C^{14} was found in the CO_2 and 0.2% in the fatty acids. With C^{14}-evenly-labeled glucose and fructose, 16% of the radioactivity was found in the CO_2 and 6% in the fatty acids. Anaerobically, although no activity was found in the CO_2, 41% of the activity incorporated by the aerobic control appeared in fatty acids; 10^{-4}M dinitrophenol decreased C^{14} incorporation from acetate-2-C^{14} into fatty acids by 43% although respiratory CO_2 production was not inhibited. With 10^{-5}M dinitrophenol, no inhibition of fatty acid synthesis and CO_2 production was observed.

Phosphatases of rat liver nuclei. MARJORIE A. SWANSON AND MARGARET C. MITCHELL.* *Dept. of Biochemistry, Bowman Gray School of Medicine, Winston-Salem, N. C.*

A preparation of nucleoprotein from rat liver nuclei was investigated for both ATPase and A-5-MPase (muscle adenylic acid phosphatase). Enzymatic depolymerization of the nucleic acid does not diminish phosphatase activity. The proteins partially freed from nucleic acid contain an ATPase with an optimum pH of around 7, which is activated by either Mg^{++} or Ca^{++}. The latter is the more effective activator. The data suggest that there may be two A-5-MPases, one activated by Mg^{++} and having an optimum pH above 8, the other activated by either Mg^{++} or Ca^{++} and having an optimum pH about 7. The response of the partially purified proteins to changes in the concentration of the various ions is different from that of the crude nucleoproteins. In all the systems studied there is a close relationship between the optimum concentrations of hydrogen and divalent metallic ions. (Aided by a grant from the American Cancer Society.)

Inhibition of liver xanthine oxidase induced by chloramphenicol. MARIAN E. SWENDSEID, Ann L. SWANSON* AND FRANK H. BETHELL.* *Thomas Henry Simpson Memorial Inst. for Med. Research, Univ. of Michigan, Ann Arbor.*

This report is concerned with an effect of the antibiotic Chloramphenicol (Chloromycetin) on an enzyme, xanthine oxidase, present in mammalian tissue. Weanling male rats were maintained on an 18% casein-dextrose diet supplemented with synthetic vitamins and 1% Chloramphenicol for a 4-week period. Food intakes were similar to those of control rats fed an identical diet without the inclusion of antibiotic, but weight gains were lower. When xanthine oxidase from livers of these rats was measured in the Warburg by the method of Axelrod and Elvehjem, O_2 uptake values were zero. Forty to 60 μl. O_2 uptake in 60 minutes/332 mg. tissue were obtained in livers from rats on control diet. Chloramphenicol added *in vitro* to liver tissue had no effect on xanthine oxidase activity. In rats, when the folic acid was omitted from the vitamin supplement in the Chloramphenicol-containing diet, the inhibition of liver xanthine oxidase could not be demonstrated. A photolytic product of folic acid, a formyl derivative, has been shown by Lowry and co-workers (*J. Biol. Chem.* 180: 399, 1949) to be a powerful *in vitro* inhibitor of xanthine oxidase. It is suggested that some metabolic product of folic acid may be present in increased amounts in Chloramphenicol-fed rats and be the responsible agent for xanthine oxidase inhibition. In support of this hypothesis, it has been found that the *in vitro* conversion of folic acid to an *L. citrovorum* growth stimulating substance in liver homogenates (method of NICHOL and WELCH, *Proc. Soc. Exper. Biol.* 74: 52, 1950) is inhibited by Chloramphenicol.

Acetate oxidation by *Escherichia coli*. H. E. SWIM AND L. O. KRAMPITZ. *Dept. of Microbiology, School of Medicine, Western Reserve Univ., Cleveland, Ohio.*

Acetate-1-C^{14} is oxidized by *Escherichia coli* anaerobically with fumarate as oxidant according to the equation: acetate + 4 fumarate + $2H_2O \rightarrow$ 4 succinate + $2CO_2$. Succinate contains all the isotope indicating recycling does not occur. The CO_2 arises from the carboxyl groups of fumarate (*Bact. Proc.*, p. 138, 1951). These facts suggested succinate synthesis from acetate by a tricarboxylic acid cycle type of mechanism rather than the Thunberg dicarboxylic acid cycle. With no recycling the succinate from acetate-2-C^{14} should contain isotope in but one methylene group, if the former cycle were operating, whereas in the latter cycle both methylene groups should be labeled. Acetate-2-C^{13} containing 53% C^{13} was employed and the relative proportions of singly and doubly labeled ethylene obtained from the methylene carbons of the succinate were determined by mass spectrometry. The validity of such analyses has been established (H. G. WOOD, *J. Bio. Chem.*, in press). The following types of ethylene are possible: doubly labeled, mass = 30; singly labeled, mass = 29; non-labeled, mass = 28. Within the limits of experimental error all the labeled ethylene was mass 29 indicating a singly labeled succinate. These results prove that the dicarboxylic acid cycle which includes an oxidative condensation of acetate to succinate does not occur under these conditions. Cell-free preparations have been prepared which oxidize acetate and other tricarboxylic acid cycle intermediates aerobically. Citrate is oxidized to α-ketoglutarate in the presence of arsenite. Other experiments with the cell-free system will be discussed which support the tricarboxylic acid cycle in this system.

Isolation and characterisation of the fractions of trypsin digested myosin. ANDREW G. SZENT-GYORGYI. (introduced by ALBERT SZENT-GYORGYI). *Inst. for Muscle Research, Woods Hole, Mass.*

Trypsin digests myosin without affecting its ATP-ase activity (GERGELY. *Federation Proc.* 9: 176, 1950). There are two reactions involved, a rapid and a slow one. The first splits the myosin molecule into two particles which have different sedimentation constants (MIHALYI, in press). Separation was achieved by precipitating one re-

peatedly at a low ionic strength. The isolated fractions were identified by their S_{20} and were found to be homogeneous in the ultracentrifuge. The fraction with the lower sedimentation constant (meromyosin-L), has the peculiar solubility of myosin, precipitating in 0.01–0.08 M KCl at neutral pH and dissolving at higher KCl concentrations. It does not have any ATP-ase activity and does not combine with actin in 0.6 M KCl. It was obtained in crystalline form. It constitutes 40–45% of the initial myosin. The fraction with the higher S_{20} (meromyosin-H), is soluble at any KCl concentration at pH 7 and precipitates at pH 5.1. Meromyosin-H can combine with the same amount of actin as the initial myosin and has the whole ATP-ase activity of the latter. It forms a complex with actin at any KCl concentration (0.01–0.6 M) and the complex is completely soluble at pH 7. The complex is dissociated into its components by a small amount of ATP regardless of whether KCl is present or not. It constitutes 55–60% of the myosin by weight.

Chromatographic homogeneity of lysozyme preparations. HARRIS H. TALLAN (introduced by WILLIAM H. STEIN). *Labs. of Rockefeller Inst. for Medical Research, New York City.*

It has been demonstrated previously (*J. Am. Chem. Soc.* 73: 2976, 1951) that a preparation of lysozyme carbonate which had been kept for a year gave two active peaks (A and B) when chromatographed on the ion exchange resin IRC-50. Further investigation has shown that freshly prepared lysozyme carbonate contains only about 7% of the faster moving peak B material. While standing at room temperature, however, the concentration of peak B material in the lyophilized preparation gradually rises and attains a value of 45% after 170 days. Simultaneously, 14% of a third active component (peak C), which moves ahead of peak B, is formed. These changes can be duplicated to some extent by exposure of lysozyme to aqueous NaOH at pH 9.0–9.5. Inactivation and precipitation also occur, however, causing variable results. These findings, together with chromatographic analyses of the isoelectric protein (which contains about 5% of B) and the chloride (3% of B and 3% of a fast-moving inactive contaminant), demonstrate that lysozyme completely free from chromatographically detectable impurities is not obtainable by the usual methods. Chromatographically and electrophoretically pure peak A lysozyme, which has previously been shown to be chromatographically indistinguishable from the enzyme occurring in whole egg white, has been prepared by the use of large columns of IRC-50. In order to isolate the enzyme, the effluent is freed of buffer by passage through a column of the mixed resins IR-120 and IRA-400 and lyophilized.

Mechanism of lipoxidase catalysis. A. L. TAPPEL,* P. D. BOYER AND W. L. LUNDBERG. *Div. of Agricultural Biochemistry, Univ. of Minnesota, St. Paul.*

Investigations which have been interpreted as supporting a chain mechanism for soybean lipoxidase catalysis (e.g., KUNKEL, *Arch. Biochem.* 30: 306, 1951) are of importance both with respect to the nature of lipoxidase action and to the possibility of enzyme action proceeding by a chain mechanism. Results to be presented show that all or nearly all the action of soybean lipoxidase on sodium or methyl linoleate is not the result of a chain reaction. In properly prepared and assayed systems the reaction velocity is directly proportional to the enzyme concentration and not to the square root of the enzyme concentration. Observations that the linoleate peroxides formed are esssentially completely conjugated and on the effects of antioxidants are not readily explainable by a chain mechanism. Antioxidants have a direct inhibitory effect on lipoxidase as well as being oxidized in the presence of linoleate. A rapid oxidation of nordihydroguaretic acid can be obtained under conditions where no linoleate is oxidized, the linoleate having a function analogous to a coenzyme. The reaction velocity approaches a maximum with increase in the substrate concentration (K_m for sodium linoleate about 2×10^{-5} M) in harmony with most other enzymic reactions. The apparent K_m for oxygen was increased at high linocleate concentrations. A simple mechanism for lipoxidase catalysis in harmony with the various experimental results to be presented involves interaction of the lipoxidase, linoleate, and oxygen with formation of a biradical which is stabilized by the enzyme and which may react with antioxidants or rearrange to give conjugated peroxides.

Microbiological assay of vitamin B_{12} in crude materials. H. L. A. TARR. *Pacific Fisheries Exper. Station, Vancouver, B.C.*

Work in two different laboratories has shown that addition of cyanide but no reducing agent to media in titrimetric assays has given similar results with cyano- and hydroxo-cobalamin. Titrimetric procedures are slow, and use of cyanide without reducing agent in Skeggs (1950) medium resulted in slow growth, while simultaneous use of reducing agent and cyanide permitted rapid growth but rarely gave the same results with the different cobalamins. Aseptic addition of both cyano- and hydroxo-cobalamin to the medium using 0.5 mg/ml. of mercaptosuccinic acid gave identical turbidimetric assay curves, while use of

ascorbic acid under such conditions had a pronounced destructive action on hydroxo-cobalamin. Sterilization in the medium normally enhanced growth with cyano-cobalamin but had a marked destructive effect on hydroxo-cobalamin. These findings are important because in crude protein materials much of the vitamin B_{12} occurs as hydroxocobalamin or similar form chromatographically (WOODRUFF AND FOSTER KH_2PO_4-paper method). When 500 mμg. of cyano- or hydroxo-cobalamin was added to 1-gm. samples of fish meal and these were autoclaved 5 minutes at 120°C., 95–110% of the added vitamin could be recovered by aseptic addition turbidimetric assay. Chromatographic separation of cyano- and hydroxo-cobalamins from such preparations (2–5 mμg.) followed by elution and combined assay gave 86–89% recovery. With this method of assay heating crude materials with a reducing agent such as mercaptosuccinic acid may cause fictitiously high recovery due to formation of a more reactive form of the vitamin. Proteolysis with enzymes often occasions formation of high concentrations of uncharacterized vitamin B_{12}-active substances.

Two dimensional paper chromatography of proteins. HENRY TAUBER AND EDWARD L. PETIT.* *Veneral Disease Exper. Lab., U. S. Public Health Service, Univ. of North Carolina, School of Public Health, Chapel Hill.*

Blood serum, several blood plasma fractions, and two hemin-containing proteins (cytochrome *c*, and crystalline catalase) have been subjected to two dimensional filter paper chromatography. Aqueous salt solutions were used as the developing media (Franklin and Quastel). A protein staining reagent, containing the fluorescent dye eosin, and methyl orange has been developed for locating the movement of proteins. Using this reagent the outlines of the patterns may be checked while damp, with the aid of ultraviolet light (Mineralight). The stained chromatograms offer a permanent record. Improvements in the two dimensional procedure will be presented. Slides of interesting and typical patterns of proteins subjected to one dimensional, and two dimensional filter paper chromatography will be shown.

Isolation of bacterial pyrogens. DAVID M. TENNENT (introduced by ROBERT H. SILBER). *Merck Inst. for Therapeutic Research, Rahway, N. J.*

Crude bacterial pyrogen concentrates prepared by ammonium sulfate precipitation of harvest supernatants of a wild coliform organism or of a *Salmonella*, grown either on agar or in liquid culture, have had considerably higher activity than that generally reported for purified fractions isolated after extraction with phenol or treatment with proteolytic enzymes. Thus it appears that the pyrogenic substance should be isolated in as near native form as possible. The crude material prepared here was analyzed, and stability studies were carried out. The effect of filtration, lyophilization and dialysis were determined. For isolation, solvent extraction was unsuccessful and precipitation with heavy metals generally unsatisfactory. Chromatography on Darco yielded better separations than on Florex or acid-washed alumina, but the greater part of the activity was not recovered. Precipitation by PH adjustment with the addition of alcohol yielded a preliminary fractionation which could be carried further by electrophoretic methods.

Enzymatic degradation of soluble bound biotin. RICHARD W. THOMA* AND W. H. PETERSON. *Dept. of Biochemistry, Univ. of Wisconsin, Madison.*

Biotin occurs in bound forms in liver (W. S. CHANG AND W. H. PETERSON, *J. Biol. Chem.*, 193: 587, 1951) and in yeast (L. D. WRIGHT *et al.*, *Science* 114: 635, 1951). The work reported here is concerned with the soluble bound biotin (SBB) contained in peptic digests of hog liver. SBB is utilizable by *Saccharomyces cerevisiae* and *Lactobacillus casei* but not by *Lactobacillus arabinosus*. It is made available to *L. arabinosus* by the action of an enzyme (called biotinidase) extractable from hog liver. The enzyme occurs also in hog kidney, chick pancreas and a purified fusarium lipase, but not in 20 other crude or partially purified enzyme preparations. Enzyme activities are compared under fixed conditions and are expressed in biotinidase units. Ammonium sulfate (1.69 M) precipitates 27% of the biotinidase of hog liver extracts substantially free of the enzymes which hydrolyze ethyl acetate, triacetin and *dl*-biotin methyl ester. No precipitation and no loss of biotinidase occurs in 10% ethanol at 0°, but at 50% all activity is lost. In contrast, 90% of the esterase which hydrolyzes ethyl acetate is precipitated by 50% ethanol without any appreciable destruction. At 0° 5% acetone causes no precipitation of biotinidase, but 10% gives a 22-fold purification and a 75% yield. The differences in solubility and sensitivity of biotinidase and esterases suggest that different agents are involved. The peptidase activity of biotinidase preparations and the stability of SBB suggest that SBB is an amide and biotinidase is an imidase.

Effect of methionine and some emulsifiers on fat absorption. H. C. TIDWELL AND MARY E. NAGLER.* *Biochemistry Dept., Southwestern Med. School, Dallas, Tex.*

The failure of Stare and co-workers (*Proc. Soc. Exper. Biol. & Med.* 75: 680, 1950) to find an in-

creased rate of fat absorption when the fat was supplemented with choline prompted a reinvestigation of our earlier study. Both a higher blood fat level and an increased rate of disappearance of the fat from the intestine of the rat were obtained by our methods when the fat was supplemented with 15–30 mg. of choline. The use of less choline and shorter absorption periods might explain our results. Further studies with supplements of methionine would indicate that it does have some effect on the rate of fat absorption. Large amounts (120 mg.) definitely depressed the rate as is true with mecholyl, prostigmin and possibly choline when given in excessive amounts. When the normal rate is given small amounts of fat, Tween 80 and similar emulsifiers do not appear to affect the rate of fat absorption as measured by the chylomicrograph or a modified Cori technique. These substances have been reported to increase the rate of vitamin A absorption and fat retention under certain conditions. Furthermore, Tween 80 and glyceryl monooleate were found to have no effect on the rate of fat absorption in young men under conditions similar to those reported by Becker *et al.* (*Gastroenterology* 14: 80, 1950). Thus normally, it appears that the fat absorption rate is not affected by exogenous emulsifiers but may be increased by substances affecting the chemical mechanism of fat absorption.

Chemical partition of dog red cell stroma. G. H. Tishkoff, F. S. Robscheit-Robbins and G. H. Whipple (introduced by Frances L. Haven). *Dept. of Pathology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Methods of isolation of normal dog red cell stroma have been studied in detail. The chemical nature of stroma has also been investigated with respect to total lipid, total phospholipid, sphingomyelin, cephalin, lecithin, total cholesterol, cholesterol ester, cerebroside, neutral fat, total protein, total iron and total hemin. The chemical pattern for the normal dog has been established. Subsequent comparison of stroma from normal dogs with stroma isolated from dogs rendered anemic by repeated bleedings has revealed alteration of the chemical pattern.

Formation of glycine and serine in plants from glycolic acid. N. Edward Tolbert and Marjorie S. Cohan (introduced by Paul B. Pearson). *Bureau of Plant Industry, Soils and Agricultural Engineering, Beltsville, Md.*

Major end products from the *in vitro* oxidation of glycolic acid by barley sap are glycine and a polymer which can be hydrolyzed by hydrochloric acid to glycine. Using carbon-14 labeled glycolic, it has been shown that the carboxy carbon of glycolic becomes the carboxy carbon of glycine and that the alpha carbon of glycolic becomes the alpha carbon of glycine. Previous work has shown that glyoxylic acid is an intermediate. Glycine is also produced *in vivo* when plants are fed carbon-14 labeled glycolic, but then more of the carbon-14 appears in serine. Products from *in vitro* utilization of glycolic acid by green barley sap, etiolated wheat sap, and barley chloroplasts and *in vivo* studies with green and etiolated wheat leaves will be discussed. Degradation data on the glycolic, glycine and serine are presented for comparison with the metabolic pathways of serine formation in animals.

L-histidine-2-C^{14}, a source of labile methyl groups with transmethylation enhanced by choline deficiency. Milton Toporek (introduced by Leon L. Miller). *Depts. of Radiation Biology and Biochemistry, School of Medicine and Dentistry, Univ. of Rochester, Rochester, N. Y.*

L-Histidine-2-C^{14} (17 mg., 7 μc.) fed to normal Wistar strain rats is a potent source of labile methyl groups in liver choline. The isolated normal rat liver perfused with whole blood utilizes histidine-2-C^{14} as efficiently as the intact animal. Otherwise normal rats on a choline-deficient diet for 10 days show greatly enhanced utilization of

Rat preparation + L-Histidine-2-C^{14}	Choline platinate disinteg/min/mM $\times 10^{-4}$	Trimethylamine platinate disinteg/min/mM $\times 10^{-4}$
Intact, normal.....	31	26
Intact, choline-deficient diet 10 days..	214	184
Perfused normal liver	25	20
Perfused cirrhotic liver.....	85	77

the 2-carbon of histidine for labile methyl group synthesis. After prolonged choline deficiency with production of experimental cirrhosis, the isolated perfused cirrhotic rat liver also manifests enhanced transformation of the 2-carbon of histidine to choline methyl groups. The data indicate that the ethanolamine carbons of choline contain approximately 15% of the total C^{14} activity of choline.

Biosynthesis of capsular streptococcal polysaccharide. Yale J. Topper and Murray M. Lipton (introduced by DeWitt Stetten, Jr.). *Divs. of Nutrition and Physiology, and Applied Immunology, Public Health Research Inst. of the City of New York, Inc.*

The biosynthesis of capsular polysaccharide, principally hyaluronic acid, by group A hemolytic streptococci grown in Dole's medium containing glucose-1-C^{14} has been studied. Comparison of the specific activities in the polysaccharide and in glucosamine derived from it revealed essentially the same abundance of isotope in glucosa-

mine and in glucuronic acid, indicating that both hexose fragments had arisen from the same source. Carbon-1 of the glucosamine had 5.4–6.4 times the specific activity of the intact hexosamine, showing direct conversion of glucose to glucosamine without skeletal rearrangement. To three parallel cultures containing identical quantities of glucose-1-C^{14} were added equimolar banks of nonisotopic glucosamine (I), glucuronic acid (II) and glucosone (III). The specific activities of the polysaccharides formed, compared with controls with no added bank, were: I, 0.40–0.52; II, 0.91–1.04; III, 0.70–0.72. The polysaccharide I was more than 10 times as radioactive as the glucosamine derived from it. These findings indicate that free glucosamine is efficiently incorporated into the bacterial polysaccharide whereas this is not the case for glucuronic acid. Glucosone can be utilized in this synthesis, possibly as a precursor of the glucosamine moiety.

Influence of folic acid, thymine and dehydroisoandrosterone acetate on formate C^{14} uptake by *Streptococcus faecalis*. JOHN R. TOTTER. *Dept. of Biochemistry, School of Medicine, Univ. of Arkansas, Little Rock.*

Streptococcus faecalis was grown for 40 hours on folic acid free medium supplemented with either folic acid (10 mμg/ml.), thymine (1.0 μgm/ml.) or dehydroisoandrosterone acetate (40 μgm/ml.). C^{14} sodium formate (20,000 cts/min/10 ml.) was added to each flask at the beginning of incubation. The organisms were harvested and washed repeatedly until no further reduction in radioactivity occurred. The folic acid grown organisms showed an activity of 290–445 cts/min/mg.; those grown on dehydroisoandrosterone 157–227 cts/min/mg.; while those grown on thymine had only 19–20 cts/min/mg. Increasing the folic acid concentration did not appear to affect markedly the uptake of C^{14} from formate unless supoptimal amounts of the vitamin were present. Growing the organisms on very low amounts of folic acid did not reduce the C^{14} fixation to a level as low as was repeatedly obtained on thymine.

Intracellular distribution of radioactivity in nucleic acid nucleotides and proteins following simultaneous administration of P^{32} and glycine-2-C^{14}. EVELYN P. TYNER,* CHARLES HEIDELBERGER AND G. A. LEPAGE. *McArdle Memorial Lab., Univ. of Wisconsin, Madison.*

Rats bearing multiple Flexner-Jobling carcinoma transplants were injected simultaneously with P^{32} and glycine-2-C^{14}. The animals were sacrificed after 2 hours and the pooled livers and tumors were separated by differential centrifugation into nuclear, mitochrondrial, microsomal and supernatant fractions. The specific activities of inorganic phosphate and free glycine were determined in each tissue. The mononucleotides from the RNA of each fraction and from the DNA isolated from the nuclei were separated by ion-exchange chromatography. The specific radioactivities of the nucleotide phosphorus and purine carbon were determined. The C^{14} specific activity of the proteins in each fraction decreased in the order microsomes, supernate, mitochondria, and nuclei and were of the same order of magnitude in liver and tumor. In all nucleotides the specific activity of both isotopes was higher in tumor than in liver. The highest specific activity of both labels in both tissues was found in the nuclear RNA fraction, followed in order by supernate, microsomes and mitochondria. The specific activities of both isotopes were higher in the DNA of tumor than of liver. Whereas the specific activity of the phosphorus in the RNA nucleotides greatly exceeded that in the DNA nucleotides, the C^{14} specific activities of the DNA and RNA purines were of the same order of magnitude. Detailed numerical data will be presented for this and other time intervals following the administration.

Oxidative properties of ascorbic acid. SIDNEY UDENFRIEND, CARROLL T. CLARK,* JULIUS AXELROD* AND BERNARD B. BRODIE. *Section on Chemical Pharmacology, Natl. Heart Inst., Natl. Insts. of Health, Public Health Service, Federal Security Agency, Bethesda, Md.*

Numerous compounds are oxidized by ascorbic acid plus oxygen, but little information is available concerning the mechanism. This report concerns the oxidation of aromatic compounds by ascorbic acid and oxygen. Fe^{++}, Fe^{+++}, Cu^{++} and Co^{++} catalyze the reaction; the catalytic effect is augmented by ethylenediamine tetraacetate, a metal complexing agent. The oxidations of tyramine to hydroxytyramine, quinoline to 3-OH quinoline, and acetanilide to p-OH acetanilide, were investigated using the following system at 37°: 0.025M substrate; 0.059M ascorbic acid; 0.005M Fe^{++}; 0.027M ethylenediamine tetraacetate; 0.1M phosphate buffer, pH 5.5; oxygen. The hydroxyl group appeared in positions having a net negative charge, suggesting replacement of ring hydrogen by OH^+. Ascorbic acid is not the primary hydroxyl donor since hydroxylation proceeds rapidly even when its concentration becomes negligible. Dehydroascorbic acid is not the oxidant since it is less active than ascorbic acid. H_2O_2 cannot be demonstrated by chemical assay and equivalent amounts of H_2O_2 act neither qualitatively nor quantitatively like ascorbic acid. However, the addition of H_2O_2 to the ascorbic acid system increases many fold the initial rate of oxidation. This suggests an organic peroxide intermediate. Several reports have implicated ascorbic acid in

aromatic hydroxylations, *in vivo*. It may be involved in these oxidations through an active intermediate such as the one indicated in these experiments.

Competitive interactions between isoleucine and valine transamination in *Escherichia coli*. H. EDWIN UMBARGER AND BORIS MAGASANIK (introduced by J. HOWARD MUELLER). *Dept. of Bacteriology and Immunology, Harvard Med. School, Boston, Mass.*

In growth experiments with isoleucine and valine requiring mutants and the wild strain (K-12) of *Escherichia coli*, a competitive antagonism between valine and isoleucine has been demonstrated (*J. Biol. Chem.* 189: 277, 287, 1950). These studies revealed that the immediate precursors of valine and isoleucine were the corresponding α-keto acids and that a similar antagonism occurred between these precursors. The final step in the biosynthesis of the amino acids has been demonstrated with dried cells as a transamination in which glutamic acid served as amino donor and pyridoxal phosphate as coenzyme. Using filter paper chromatography for the quantitative determination of keto acids in mixtures (*Abstracts, Am. Chem. Soc.*, 119th meeting, April 3, 1951, 18c), it has also been possible to study the reverse reactions using valine and isoleucine as amino donors. The apparent dissociation constants of the enzyme complex with coenzyme, amino donor, and amino acceptor were determined and found in each case to be different for the two systems. The isoleucine-α ketoglutarate transamination was the more rapid reaction. When both amino acids were present and undergoing transamination simultaneously, a competitive antagonism was observed in which the valine-α ketoglutarate transamination was strongly inhibited by isoleucine. Valine, which inhibits the growth of strain K-12 in the absence of isoleucine, had very little inhibitory effect on isoleucine-α-ketoglutarate transamination. These and other observations will be discussed as examples of the non-hormonal regulation of biosynthesis and growth in bacteria (DAVIS. *J. Clin. Inv.*, 29: 808, 1950).

Time course of lipid labeling following single injections of C^{14} acetate. J. T. VAN BRUGGEN,* C. K. CLAYCOMB,* T. T. HUTCHENS* AND E. S. WEST. *Dept. of Biochemistry, Univ. of Oregon Med. School, Portland.*

The time course of C^{14} incorporation into lipid has been studied by determining specific and total activities in fatty acid and cholesterol fractions of liver, skin, carcass, gut and brain plus spinal cord from pairs of rats sacrificed at 15, 30, 60, 90 and 120 minutes after an intraperitoneal injection of carboxyl labeled acetate. Respiratory $C^{14}O_2$ collection and assay indicate that the tracer was metabolically available to all groups. The 0 time and 7-minute groups were studied similarly but without CO_2 collections. Low, but significant, C^{14} incorporation into lipid was found in the 0 time rats. The percentage of tracer incorporated into fatty acid and cholesterol by the 7-minute animals was only slightly less than the 15-minute incorporation figure. The percentage incorporation into fatty acid and cholesterol fractions at 30, 60, 90 and 120 minutes were variable but showed no general increase or decrease, the averages of these values being only slightly higher than the 15-minute incorporation figures. The data indicate an early rapid rate of fatty acid and cholesterol labeling which brings the lipid C^{14} incorporation to a plateau within 15–30 minutes after injection of the tracer. We have obtained no evidence for a rapidly metabolizing liver fatty acid fraction such as has been reported in mice by Hevesy, Ruyssen and Beekmans (*Experientia* 7, 144–6, 1951).

Study of *in vitro* action of aureomycin on oxidative phosphorylation in animal tissues. J. C. VAN METER,* A. SPECTOR,* J. J. OLESON AND J. H. WILLIAMS.* *Lederle Labs. Div., American Cyanamid Co., Pearl River, N.Y.*

In a preliminary report (*Science* 113: 273, 1951) from this laboratory, aureomycin was shown to strongly inhibit respiration in rat liver homogenates. These results are at variance to those of Loomis (*Science* 111: 474, 1950) who found only inhibition of phosphorylation but not of oxidation. We have found that not only the respiration of homogenates is inhibited by aureomycin but also that of washed rat liver mitochondria. Using the suspending medium of Kielly and Kielly (*J. Biol. Chem.* 191: 485, 1951) it was found that aureomycin had only the effect reported by Loomis. However if adenosine triphosphate (Schwarz) was substituted for adenylic acid, the inhibitory effect of aureomycin on oxidation appeared. Reduction of the inorganic phosphate to 30 micromoles and below per flask accentuates this effect. Further investigation of the relationship of aureomycin to ATP on this effect is in progress.

Competitive inhibition of uricase by xanthine; an assay for xanthine oxidase activity in the presence of uricase by the *in vitro* production of uric acid. JOHN F. VAN PILSUM (introduced by SHERMAN R. DICKMAN). *Dept. of Biochemistry, Univ. of Utah, College of Medicine, Salt Lake City, and Dept. Biochemistry, Univ. of New York, College of Medicine, Brooklyn.*

Incubation of rat liver homogenate with relatively large amounts of sodium xanthine resulted in an easily measurable production of uric acid. Brown's colorimetric method was used. Because

rat liver contains a high uricase activity, the urate production was a result of either *1*) an inhibition of the uricase by the xanthine or *2*) by the inability of the uricase to destroy all of the urate formed by the xanthine oxidase. The first explanation was substantiated. An incubation system was devised that contained sufficient sodium xanthine to inhibit the uricase present, but which would not inhibit the xanthine oxidase itself. One ml. of a 10% liver homogenate (in phosphate buffer pH 7.4) was mixed with 6 ml. of aqueous mono sodium xanthine (containing 7 mg/ml.) and 3 ml. of water and incubated one hour at 37°C. The amount of uric acid in the system before and after incubation was determined. The uric acid production by liver tissue from both normal and protein depleted rats agreed with the oxygen uptake as measured by the Axelrod and Elvehjem manometric method. The inhibition of a purified preparation of uricase by xanthine was overcome or reversed by increasing the concentration of urate in the system. A Lineweaver-Burke plot of V/V_I vs (I) indicated the inhibition is competitive.

Growth requirements of *Paramecium aurelia*, var. 4, stock 51.7 (s) in a medium free of other living organisms. W. J. VAN WAGTENDONK, C. A. MILLER,* AND R. L. CONNER.* *Dept. of Zoology, Indiana Univ., Bloomington, Ind.*

A medium for the pure culture of *Paramecium aurelia* (sensitives) which supports 2–3 fissions per day has been developed. Its principal components are: *1*) a salt solution (NaCl, 150 mg.; KCl, 25 mg.; $CaCl_2 \cdot 2H_2O$, 25 mg.; $MgSO_4 \cdot 7HO$, 50 mg.; K_2HPO_4, 100 mg.; $Fe(NH_4)_2(SO_4)_2 \cdot 6_2O$, 5 mg.; water, 1 l.); *2*) a mixture of water-soluble vitamins (thiamin, 5 γ; riboflavin, 2.5 γ; niacin, 18 γ; Ca-pantothenate, 5 γ; pyridoxine, 5 γ; pyridoxal, 2.5 γ; pyridoxamine, 2.5 γ; folic acid, 2.5 γ; biotin, 1.25 γ; choline, 5 γ; final concentration/ml); *3*) the non-dialyzable fraction of an autoclaved and filtered yeast autolyzate, prepared from air-dried fresh bakers' yeast (final concentration 0.75 mg/ml.); *4*) the non-dialyzable fraction of proteose peptone (final concentration 7.0 mg/ml.); *5*) a growth factor for *Paramecium aurelia* obtained from lemon juice. The components 3, 4, and 5 are essential. No growth occurs when component 2 is omitted from the medium, but the essentiality and the minimal amount of each of the constituents have not yet been established. The factor obtained from lemon juice is heat and acid stable and is slowly destroyed by autoclaving at pH 11. The factor is soluble in ether, benzene, ethanol, butanol and amyl alcohol. The factor is widely distributed in plant material, but its presence in animal tissues could not be demonstrated. The factor cannot be replaced by α-lipoic acid.

Conjugated corticoids. ELEANOR H. VENNING. *McGill Univ. Clin., Royal Victoria Hosp., Montreal, Canada.*

A comparison of results obtained by chemical and biological assays of urinary corticoids released from conjugation by acid and β glucuronidase hydrolysis has been made. By the chemical method, 10–20 times greater amounts of formaldehydogenic steroids can be obtained after hydrolysis with β glucuronidase than by extraction of the urine at pH_1. The biological assay shows only an increase of 2–4 times the activity. The conjugated corticoids that can be extracted from the chloroform extract with sodium bicarbonate also show activity. Values obtained on normal individuals, patients with Addison's disease, adrenal tumour and liver disease will be presented.

Metabolism of fetal tissues *in vitro*. CLAUDE A. VILLEE. *Depts. of Biological Chemistry and Obstetrics, Harvard Med. School, Boston, Mass.*

Tissue slices, prepared from fetuses and placentas obtained at therapeutic interruptions of pregnancy, were incubated in Warburg vessels in a Ringer-phosphate medium containing C^{14}-labeled glucose, pyruvate, acetate, oxalacetate or glycerol. Oxygen uptake, substrate utilization, and glycogen, carbon dioxide and lactate production were measured. Twenty fetuses, aged 9–23 weeks, were used. By the 8th week of development, fetal tissues are well differentiated biochemically and show the patterns of carbohydrate metabolism characteristic of corresponding adult tissues: liver has a high glycolytic rate, brain has the highest rate of glucose utilization and the lowest rate of pyruvate utilization of the 6 tissues studied. Heart has the highest rate of pyruvate utilization and lactate production. Brain metabolizes pyruvate to CO_2 but uses acetate only slowly; in contrast, liver metabolizes acetate at least as well as pyruvate. Oxalacetate is decarboxylated rapidly by all tissues and the presence of unlabeled oxalacetate decreases the amount of radiopyruvate metabolized to CO_2. It was possible to observe the development of the enzyme, glucose-6-phosphatase: it appears at 11 weeks in liver and at 16 weeks in kidney. The placenta has strong glucose-6-phosphatase activity early in development but this gradually decreases and is completely absent at term. Thus it appears that before 12 weeks the placenta acts as a 'liver' to store glycogen and regulate the fetal blood glucose level, and that this ability is lost when the fetal liver becomes active.

Phosphorylation coupled to the photochemical reduction of DPN by spinach grana. WOLF VISHNIAC (introduced by S. OCHOA). *Dept. of Phar-*

macology, New York Univ. College of Medicine, New York City.

As previously reported (*Nature* 167: 768, 1951) illuminated grana from spinach chloroplasts catalyze the reaction TPN (or DPN) + $H_2O \xrightarrow{(light)} TPNH_2$ (or $DPNH_2$) + ½ O_2. The above reaction has been coupled to the reductive carboxylation of pyruvate and α-ketogutarate to malate and isocitrate, the reduction of pyruvate to lactate, the reductive condensation of 3-phosphoglycerate to fructose diphosphate, and other biochemical reductions (*J. Biol. Chem.*, in press). These reactions may participate in photosynthesis. ATP is a stoichiometric reactant in the reduction of 3-phosphoglycerate and the question arises how the high-energy bond of ATP is generated in photosynthesis. Lehninger's demonstration that the oxidation of $DPNH_2$ by liver mitochondria generates high-energy phosphate (*J. Biol. Chem.*, 190: 345, 1951) suggested the following experiments: Grana from spinach leaves were incubated aerobically with DPN, rat liver mitochrondria, ATP, and $P^{32}O_4^{=}$ in the light and in the dark. After 15 minutes the ATP was separated by ion exchange chromatography and the radioactivity of its labile phosphate groups determined both directly and after transferring to glucose with hexokinase and myokinase. About 3 times more radiophosphate was incorporated in ATP in the light than in the dark. These results suggest that no special photochemical mechanism need be postulated for the formation of high-energy phosphate in photosynthesis; phosphorylation may occur through coupling with the oxidation of photochemically reduced pyridine nucleotides. Bonner (*Proc. Nat. Acad. Sci.*, in press) recently found that plant mitochrondria also carry out oxidative phosphorylation.

Bioautographic studies on the *Lactobacillus bulgaricus* factors. JAMES C. VITUCCI,* BRIAN L. HUTCHINGS AND JAMES H. WILLIAMS.* *Lederle Labs. Div., American Cyanamid Co., Pearl River, N.Y.*

Bioautographic studies showed that an unidentified growth factor(s) (designated LBF) required by several strains of *Lactobacillus bulgaricus* exists in a number of chromatographically distinct forms in natural materials (C. L. LONG, and W. L. WILLIAMS, *J. Bact.* 61: 195, 1951). The identity of the fastest moving natural form of LBF in an *A. gossypii* concentrate with a synthetic material (Pantethine) was postulated on the basis of similar biological activity and chromatographic behavior (E. E. SNELL *et al.*, *J. Am. Chem. Soc.* 72: 5349, 1950). In all chromatographic investigations on LBF, water-saturated n-butanol was the mobile phase used almost exclusively. Recently, however, the dissimilarity of Pantethine and the fastest moving form contained in a purified *B. subtilus* concentrate was demonstrated by the use of several different solvent systems in chromatographic studies (J. C. VITUCCI *et al.*, *Arch. Biochem.*, in press). The use of these alternate solvent systems (butanol, ethyl acetate, amyl alcohol and chloroform-pet. ether—9:1) in chromatographic studies of different source materials of LBF revealed the following: *1*) Employing butanol as the mobile phase, Pantethine has the same Rf value as the fast moving form contained in purified concentrates prepared from *B. subtilus*, *B. megatherium* and *A. gossypii* and one of the forms resulting from intestinal phosphatase treatment of coenzyme A. *2*) Use of the alternate systems demonstrated that Pantethine is non-identical with any of the natural fast moving forms. *3*) Intestinal phosphatase treated CoA contains forms not identical with Pantethine or any of the natural forms studied.

Enzymatic degradation products of ribonucleic acid. ELLIOTT VOLKIN* AND WALDO E. COHN. *Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.*

The 5′ nucleotides of ribonucleic acids (RNA), previously isolated in small amounts from intestinal phosphatase digests (COHN AND VOLKIN, *Nature* 167: 483, 1951) have now been recovered in about 60% yield with the use of purified snake venom diesterase. The remainder is accounted for as purine nucleosides and pyrimidine diphosphates in approximately equivalent amounts. The latter have been characterized as the 2′, 5′ and/or 3′, 5′ diphosphates of cytidine and uridine. Since the same RNA's hydrolyzed with alkali yield exclusively 2' and/or 3' nucleotides, the major internucleotide link is considered to be 2′, 5′ and/or 3′, 5′. The next question, with respect to the structure of RNA, is that of sequence of nucleotides and the structure of branch points. An excellent system for such a study is the pancreatic ribonuclease digest, where the limit reaction products consist in a large variety of polynucleotides in addition to 70% of the pyrimidines as *b* nucleotides. It has been found possible to fractionate such a mixture by ion-exchange. The purified polynucleotide products are then characterized by analysis with the above-mentioned enzymes, together with others of different specificities, e.g. Schmidt's prostatic phosphatase, which hydrolyzes only end-group phosphoryl groups, and the spleen nuclease of Maver and Greco (*J. Biol. Chem.* 181: 861, 1949), which we find to liberate only *b* nucleotides (and *a* guanylic) from intact RNA. From analyses of this type, an attempt can be made to reassemble the degradation products into a structure representative of intact RNA.

Enzymatic splitting and synthesis of glutathione (GSH). HEINRICH WAELSCH, PAUL J. FODOR* AND ALEX MILLER.* *N. Y. State Psychiatric Inst. and Dept. of Biochemistry, Columbia Univ., New York City.*

In continuation of the study of the biological role of glutamine, the action of the amide in activating the enzymatic removal of the glutamic acid portion of GSH as measured by the Sullivan reaction was investigated. Glutamine was the most potent activator of a partially inactivated enzyme preparation from kidney; γ-ethylglutamate was slightly less active than glutamine, while various amino acids showed a minimal effect. The amide configuration is, therefore, not essential for the activation of the enzyme system. The activation of the hydrolysis of GSH by glutamine and other amino acids is interpreted as the transfer of the glutamic acid radical of GSH to glutamine or other amino acids with the formation of the corresponding γ-glutamylpeptides. The residual activity of the non-activated enzyme is probably caused by a transfer of the γ-carboxyl group of the GSH-glutamic acid to its own α-amino group with the formation of pyrrolidon carboxylic acid. γ-Glutamylglycine or γ-glutamylglutamic acid inhibited strongly the glutamine activated hydrolysis of GSH while α-glutamylglutamate was inactive. On the basis of this and additional experimental evidence, it appears that the amino acid-activated enzymatic removal of the glutamic acid portion of GSH is a reversible process and that the incubation of γ-glutamylpeptides with cysteinylglycine leads to the resynthesis of GSH by an exchange reaction.

Electrophoresis of soluble cytochromes. W. W. WAINIO, BERTRAM EICHEL* AND S. J. COOPERSTEIN.* *Bureau of Biological Research, Rutgers Univ., New Brunswick, N. J. and Dept. Anatomy, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

Preparation 0–4, made by high-speed centrifugation following the addition of 4% of sodium desoxycholate to an insoluble heart muscle preparation (*J. Biol. Chem.* 173: 145, 1948), has been subjected to electrophoretic analysis. Three prominent boundaries are noted at 100 minutes (pH 7.28, $\Gamma/2 = 0.1$) The components migrate toward the cathode at 4.0, 11.3, and 21.2×10^{-5} cm² volts^{-1} sec^{-1}, respectively. Cytochrome oxidase is *tentatively* identified as being a part of the fastest moving component comprising 15% of the protein and cytochrome *c* as being a part of the slowest moving component comprising 25% of the protein.

Citrovorum factor deficiency in rats and influence of antibiotics on growth. HARRY A. WAISMAN, JOAQUIN CRAVIOTO-M.* AND HENRY G. PONCHER.* *Dept. of Pediatrics, Univ. of Illinois College of Medicine, Chicago.*

The ability of small amounts of citrovorum factor to counteract the 'folic acid' deficiency induced by aminopterin has suggested that the folic acid antagonists are in all probability citrovorum factor antagonists as well. More folic acid than citrovorum factor is necessary to counteract known quantities of the antagonist. Aureomycin was able to overcome folic acid and/or citrovorum factor deficiency induced by aminopterin. The mode of action is presumed to be that the antibiotic alters the flora so that favorable bacteria either are encouraged to synthesize more of this factor or that deleterious bacteria are prevented from utilizing this substance for their own growth. Further data have been obtained on the role of other antibiotics in the folic acid and/or citrovorum factor deficiencies when these latter substances were either omitted from the diet or when the antagonists were used. It has been found that penicillin, dihydrostreptomycin, chloromycetin, and terramycin, unlike aureomycin, have been ineffective in counteracting the aminopterin-induced deficiency. In the presence of a diet adequate in all factors except folic acid, aureomycin was able to give increased growth above that obtained when citrovorum factor was added. The ability of other antibiotics to stimulate growth on a folic acid deficient diet has been investigated to determine whether the action of all antibiotics is uniform. The role of these substances in stimulating growth beyond that expected on a diet complete in all known vitamins suggests that additional growth factors are made available to the host by the intestinal bacteria.

Iodopsin. GEORGE WALD, PAUL K. BROWN* AND PATRICIA H. SMITH.* *Biological Lab., Harvard Univ., Cambridge, Mass.*

The first photosensitive pigment of cone vision, iodopsin, was extracted in 1937 from chicken retinas, together with rhodopsin from the rods (Wald). The absorption spectrum of rhodopsin is maximal at 500 mμ, that of iodopsin further to the red at 562 mμ. For this reason the spectral sensitivity of the eye shifts toward the red as one goes from dim to bright light, from rod to cone vision. For this reason also iodopsin bleaches in red light, leaving rhodopsin unaltered. Its bleaching, like that of rhodopsin, yields as products retinene$_1$ (cf. Bliss) and a specific protein. Since chicken retinas contain retinene reductase, the enzyme which with cozymase reduces retinene to vitamin A, this reduction presumably occurs in cones as in rods. The carotenoids of the rhodopsin and iodopsin systems are therefore identical; only the proteins are different. The cone protein can be called *photopsin*, to distinguish it from the

scotopsins of the rods. From bleached chicken retinas a colorless mixture of scotopsin and photopsin can be extracted. When this is incubated in the dark with retinene, it synthesizes a mixture of rhodopsin and iodopsin. Iodopsin, however, forms much faster than rhodopsin, probably accounting for the fact that dark adaptation is much more rapid in cones than rods. To synthesize iodopsin requires a specific cis-isomer of retinene, the same as is needed to synthesize rhodopsin. The bleaching of iodopsin, however, like rhodopsin, yields an inactive isomer, apparently all-trans retinene. For cone vision to continue, therefore, the retinene or vitamin A released from iodopsin must be isomerized, or the active cis-isomer of vitamin A must be continuously supplied from the circulation. (This research was supported in part by the Medical Sciences Division of the Office of Naval Research).

Changes in rat tissue water and chloride content following a thermal burn. JAMES M. WALKER, MARIE T. BARNES, MORTON GLASSMAN, AVIS R. WALKER (introduced by HARRY M. VARS). *Harrison Dept. of Surgical Research, Schools of Medicine, Univ. of Pennsylvania, Philadelphia.*

Tissues of male white rats (Wistar strain) were analyzed for water and chloride content. Rats were scalded under ether anesthesia, producing back burns comprising 32% of body area, as described by McCarthy. Four groups, of 10 rats each, were studied; normal-unburned controls; burned-untreated rats; burned rats, infused with 10% of body weight of 1.4% sodium chloride; burned rats, infused intravenously with like amounts of 0.88% polyvinylpyrrolidone in 0.9% sodium chloride. Water content of burned skin and the underlying muscle increases, as anticipated. Significant increase in water was also found in adrenal glands, liver and kidney. Chloride content of tissues changed to a greater extent than water content. Changes were more marked in burned-treated animals than in burned-untreated. Significant chloride increments were found in gluteus medius muscle, spleen, gastric cardia and pylorus, and in small bowel. Chloride decreased in kidney, liver, adrenal gland and lung. Gluteus medius muscle and gastric cardia contained more chloride following treatment. Gastric pylorus showed less increase in chloride, and spleen chloride values were normal in treated animals. Lung and brain tissue lost chloride, even after treatment. This study shows that marked changes in tissue chloride occur in tissues not directly affected by the burn, and that shifts in tissue water are of insufficient magnitude to account for observed changes in tissue chloride content. (This work was done under contract number DA-49-007-MD-88 between the University of Pennsylvania and the Department of the Army.)

Simultaneous separation and determination of purines, pyrimidines and amino acids in hydrolysates of bacterial cells. J. S. WALL,* P. W. WILSON AND R. H. BURRIS. *Depts. of Biochemistry and Bacteriology, Univ. of Wisconsin, Madison.*

The ion exchange methods of Cohn (*Science* 109: 377, 1949) and Stein and Moore (*Cold Spring Harbor Symp. Quant. Biol.* 14: 179, 1950) have been combined and extended to permit the isolation and determination of amino acids, purines, pyrimidines and ammonia from a single column. Photosynthetic bacteria which had fixed N_2^{15} were hydrolyzed with acid, and a portion of hydrolysate containing 60–120 mg. N was applied to a column of Dowex 50, 55 cm. x 5.5 cm. Fractions of the HCl eluate were analyzed for amino acids with ninhydrin and for purines and pyrimidines by observing their absorption at 260 mμ. Uracil and thymine are resolved and are eluted with 1.5 N HCl before aspartic acid. Xanthine is eluted with 2.5 N HCl between valine and proline, and cystosine follows cystine. A shift to 6 N HCl after lysine is removed gives better resolution of arginine, guanine, phenylalanine and adenine (eluted in that order) than does 4 N acid. Uracil, thymine, xanthine, cytosine, guanine and adenine found in hydrolysates of *Chlorobacterium sp.* and *Chromatium sp.*, were identified by their position of elution, absorption spectra and by paper chromatography. The amino acids accounted for 90.8%, and purines and pyrimidines for 6.2% of the total N of the hydrolysate of *Chlorobacterium* cells. The distribution of N^{15} in amino acids, purines and pyrimidines will be discussed.

Pyruvate and acetate metabolism in bakers' yeast. CHIH H. WANG,* RICHARD C. THOMAS,* ROBERT F. LABBE,* BERT E. CHRISTENSEN,* AND VERNON H. CHELDELIN. *Dept. of Chemistry, Oregon State College, Corvallis.*

Studies in this laboratory have aimed at the comparative behavior of acetate and pyruvate as precursors of amino acids in yeast. $CH_3C^{14}OOH$ and $CH_3C^{14}OCOOH$ were administered as sole carbon sources to Fleischmann's bakers' yeast that had been previously grown in malt extract and glucose. Acetate was utilized (aerobically) about one third as rapidly as pyruvate, under either aerobic or anaerobic conditions. The harvested yeast samples were hydrolyzed, and the amino acids isolated by ion exchange resins. To date, aspartic acid, glutamic acid, tyrosine, arginine, histidine and lysine have been isolated from 3 yeast samples. Preliminary degradation of these amino acids revealed that whereas from

$CH_3C^{14}OOH$ the activity in aspartic and glutamic acids was located exclusively in the carboxyl groups, with $CH_3C^{14}OCOOH$ as substrate, 54% of the radioactivity was located at the amino carbon of aspartic acid. Forty-five per cent of the glutamic acid activity was found in the middle 3 carbons. In tyrosine from pyruvate, the carboxyl and alpha carbons contained 7% and 34%, respectively, of the total activity, compared to 40% for the carboxyl and 16% for the alpha carbon in acetate tyrosine. The results from acetate yeast were in good agreement with those reported by Ehrensvärd and co-workers, and suggested a C_2-C_2 condensation for the formation of C_4 acids. From pyruvate, a C_3-C_1 condensation, such as the Wood-Werkman or 'malic' type, apparently operated to a major extent.

Lipoproteins of cellular nuclei. TUNG-YUE WANG,* M. JOSEPH CARVER,* ROBERT H. RAMSEY,* ARNOLD J. FUNCKES* AND LLOYD E. THOMAS. *Dept. of Biochemistry, Univ. of Missouri, Columbia.*

Proteins, insoluble in water or neutral salt solutions, but soluble in alkaline solutions, have been found in whole tissues and in cellular nuclei by various workers. These proteins precipitate at about pH 6 when their alkaline solutions are acidified. In studying proteins of this nature, we have found that they contain firmly bound lipids, which are removable by hot alcohol, but not by ether. The lipo-protein complex of the nuclei of rat liver, calf liver, ox spleen and chicken erythrocytes was found to contain, in each case, approximately 10% lipid (which gives positive tests for phospolipid and cholesterol) and preliminary work indicates that, in the case of rat liver nuclei, the complex contains about 0.8% calcium. The purified lipoprotein fractions of rat liver nuclei and calf liver nuclei each have only one component as determined by electrophoresis and that of calf thymus nuclei has two components. As a rough approximation, the lipoprotein of rat liver nuclei and calf liver nuclei constitutes, in each case, 20–30% of the total dry weight of the nuclei. (The dry weight used for this calculation is the weight of nuclei isolated by a modified Behrens method. The lipoprotein is about 50% of the dry weight of nuclei prepared by the Dounce method.)

Modified 1-stage prothrombin method controlling thromboplastin and other variables. ARNOLD G. WARE AND ROBERT STRAGNELL.* *Depts. of Biochemistry and Medicine, Univ. of Southern California School of Medicine and Lab. Div., Los Angeles County Hosp., Los Angeles.*

An aliquot of prothrombin-free beef plasma has been used to control accessory clotting factors in a modified 1-stage prothrombin method which employs 1–10 dilution of the test plasma to improve accuracy and to eliminate the effects of inhibitors. Variability in thromboplastin reactivity has been controlled with dried-stable prothrombin standards. Reconstitution of weighed aliquots of the dried standards permits rapid evaluation of thromboplastin preparations yielding an approximate linear relationship between the logarithms of prothrombin concentration and time. Freshly drawn plasmas may develop an apparent increase in prothrombin which occasionally rises to 200% of the original level during a 3- to 5-hour period at room temperature. These changes in prothrombin activity have been prevented by using a special anticoagulant consisting of a mixture of oxalate and heparin. The concentration of the latter is adjusted so that it has no clot inhibiting effect when the test plasma is diluted. Using this anticoagulant, the whole plasma appears to maintain constant activity for many hours at ordinary temperatures even when allowed to remain in contact with cellular elements. The test is primarily a measure of prothrombin but also is affected significantly by the stable accelerator(s) present in serum (SPCA, convertin, factor 7). Since the coumarin anticoagulants appear to depress the serum accelerator precursor in addition to prothrombin the test is theoretically (and practically in our hands) ideal for control of coumarin therapy.

Rate of appearance of dietary cholesterol in blood. IRVING WARNER (introduced by R. GORDON GOULD). *Rush Dept. of Biochemistry, Presbyterian Hosp., and Dept. of Biological Chemistry, Univ. of Illinois College of Medicine, Chicago.*

Dogs, rabbits and terminal patients were fed single, small doses of C^{14}-labeled cholesterol and the specific activity of free and total blood cholesterol followed for several weeks. The approximate dosage in the human was 2.2 mg/kg. body weight and the specific activity was about 0.25 microcuries/mg. It was given to humans and dogs dissolved in vegetable oil with added phospholipids and to rabbits in amorphous form suspended in water. The maximum specific activity values of both free and esterified cholesterol in blood were reached by humans in about 2 days, by dogs in 1–1.5 days and by rabbits in 5–6 days. Biggs and Kritchevsky (*Circ.* 4: 34, 1951) found 2 days for a normal rabbit fed labeled cholesterol dissolved in vegetable oil; this difference may be due to slower absorption of the amorphous cholesterol. In both humans and dogs free blood cholesterol had a higher specific activity than esterified during the first 3 days, whereas in rabbits, esterified was higher than free as previously reported by Biggs and Kritchevsky. Half-turnover times as determined from the linear portions of the log

specific activity vs. time plots were 8 days for humans, and about 11–12 days for both rabbits and dogs.

Effect of 2-aminofluorene on the utilization of S^{35} DL-methionine. A. W. WASE,* J. B. ALLISON AND J. F. MIGLIARESE.* *Bureau of Biological Research, New Brunswick, N. J.*

These experiments involved both *in vitro* and *in vivo* studies of the uptake of S^{35} DL-methionine by the livers of rats. Livers from normal rats were sliced and perfused in buffered salt solution containing S^{35} DL-methionine. The system was incubated at 37°C for 2 hours. Similar systems were used including 2-aminofluorene at various concentrations, with and without riboflavin. The 2-aminofluorene inhibited the uptake of methionine by the liver slices. Riboflavin alone had no effect upon the uptake but at proper concentrations this vitamin prevented the inhibition of the carcinogen. Adult male Wistar rats were fed 0.03% of 2-aminofluorene in the diet for 56 days. At the end of this experimental period each animal was given 3 mg. S^{35} DL-methionine intraperitoneally. The uptake and disappearance rates of S^{35} DL-methionine in the livers were more rapid in the rats fed carcinogen than in the controls. Previous studies had demonstrated that 2-aminofluorene depleted certain protein stores of the animal. Depletion of liver proteins in general increased the rate of uptake of S^{35} DL-methionine markedly when this uptake was calculated in terms of liver nitrogen.

Photoöxidation of chymotrypsin in presence of methylene blue. LEOPOLD WEIL AND A. R. BUCHERT.* *Eastern Regional Research Lab., Philadelphia, Pa.*

The photoöxidation of crystalline chymotrypsin mediated by visible light in the presence of traces of methylene blue was investigated. The changes brought about by this procedure in amino acid composition and physical properties of the enzyme were correlated with its catalytic activities. The photoöxidation of the enzyme at pH 7.4 produced a parallel decrease in both proteinase and esterase activities, reaching complete inactivation at 4 mole of O_2-uptake per mole of enzyme. At this point only 1 mole of histidine out of the total of 2, and 2.4 mole of tryptophan out of the total of 6 were photoöxidized, while no changes in tyrosine, methionine and cystine contents could be observed within the enzyme molecule. No changes in solubility and viscosity occurred during the photoöxidation of the enzyme, thus indicating the absence of denaturation during the reaction. In the light of these observations the possible role of histidine or tryptophan or both of these amino acids in enzymatic activity has been considered. Controlled photoöxidation of β-lactoglobulin prior to hydrolysis with chymotrypsin reduced the available peptide bonds for this enzyme.

Metabolic block in minced snail tissues. EUGENE C. WEINBACH (introduced by LESLIE HELLERMAN). *Natl. Inst. of Health, Bethesda, Md.*

During a study, in connection with the program on the action of molluscacides, of the intermediary metabolism of the schistosome-transmitting snail, *Australorbis glabratus*, it was observed that minced tissues of this invertebrate produce significant quantities of pyruvic acid proportional to the time of aerobic incubation. Intact, living snails did not excrete pyruvic acid nor did pyruvate accumulate in their tissues. This indicated efficient utilization. Evidence that pyruvate removal in the intact organism is via a mechanism suggestive of the Krebs cycle was obtained with experiments employing aqueous, fortified homogenates; activity was found for cytochrome oxidase, fumarase, malic and succinic dehydrogenases. The addition of ATP, DPN, and cocarboxylase to the minced tissues did not expedite removal of pyruvate. The metabolic block is not due to loss, during the mincing process, of electron carriers: large amounts of added cytochrome-*c* and methylene blue *increased* rather than decreased the formation of pyruvic acid. Fluoroacetate, transaconitate and malonate had little effect on the endogenous respiration and keto acid formation by the mince, while iodoacetamide markedly suppressed both. Presumably, the terminal oxidation process was disrupted while glycolytic breakdown in its aerobic phase was unimpaired or stimulated. Incubation of the mince under anerobic conditions was accompanied by the increased formation of lacetate and nonappearance of free pyruvate. Experiments employing a variety of media indicated that the metabolic block is independent of ionic strength, inorganic ions, and pH (over the range studied).

Metabolism of tartaric acid. THEO J. WEISS AND PAUL M. RUOFF (introduced by DAN A. RICHERT). *Dept. of Chemistry, Syracuse Univ., Syracuse, N. Y.*

The metabolic pathway for the transformation of tartaric acid by *Aspergillus niger* was studied using the paper chromatographic technique of Lugg and Overell (*Australian J. Sci. Res.* 1A: 98, 1948). The culture medium consisted of 1% tartaric acid plus inorganic salts in distilled water. Phosphorylation of D-tartaric acid was apparent since a phosphotartaric acid was found in the medium and in the washed mycelium. A search was made for other intermediates. L- and meso-tartaric acids were apparently inert, since they neither supported growth alone, nor

inhibited it when glucose was added to the medium. Growth in the presence of DL-tartaric acid was compared with the growth when D-tartaric acid was used alone. Trans-epoxysuccinic acid, found by Sakaguchi (*Chem. Abstr.* 33: 7339, 1939) in *Monilia formosa* and *Penicillium viniferum*, did not support the growth of *A. niger*, although it is readily hydrolyzed to tartaric acid in boiling water. It was also found to inhibit growth when added to a glucose medium. D-tartaric acid did not affect this inhibition. The effect of the cis-epoxysuccinic acid is similar. *A. niger* converts dihydroxymaleic acid to both tartaric acid and phosphotartaric acid. No evidence of tartaric acid or phosphotartaric acid was found, however, when the cultures were grown on glucose, succinic acid or fumaric acid.

Metabolism of ethanolamine. ARTHUR WEISSBACH introduced by EDGAR G. MILLER, JR.). *Dept. of Biochemistry, Columbia Univ., New York City.*

In an effort to determine the nature of the intermediates involved in the conversion of the α-carbon of glycine to 1-carbon units (cf. WEISSBACH AND SPRINSON, *Federation Proc.*, 10: 268, 1951) it was thought desirable to investigate the metabolism of ethanolamine labeled in both carbon atoms ($C^{13}H_2OH$-$C^{14}H_2NH_2$). Following the administration of this compound to a pigeon the isolated uric acid contained most of the C^{14} in carbon 4 and C^{13} in carbon 5. Since carbons 4 and 5 are derived from glycine it would appear that $C^{13}H_2OH$-$C^{14}H_2NH_2$ was converted to $C^{13}H_2NH_2$-$C^{14}OOH$ and did not give rise to a 1-carbon unit. Similar labeling was found in urinary hippuric acid following feeding of the labeled ethanolamine to rats. A reasonable sequence of reactions for this conversion would be: ethanolamine → glycolaldehyde → glycolic acid → glyoxylic acid → glycine. In this scheme some of the intermediates may occur as phosphorylated derivatives. The oxidation of ethanolamine to glycine establishes a cycle of reactions for the oxidation of glycine and serine to CO_2 without mediation of the tricarboxylic acid cycle: glycine → serine → ethanolamine + CO_2 → glycine.

Crystalline disaccharide from hyaluronic acid. BERNARD WEISSMANN (introduced by KARL MEYER). *Dept. of Medicine, Columbia Univ. College of Physicians and Surgeons, and Edward Daniels Faulkner Arthritis Clinic of Presbyterian Hosp., New York City.*

In a recent communication (RAPPORT, WEISSMANN, LINKER AND MEYER, *Nature* 168: 996, 1951), the isolation of a crystalline glucuronidoglucosamine, 'hyalobiuronic acid', from hyaluronic acid was reported. Work on this disaccharide has been continued. Among the more important derivatives which have been prepared are a crystalline heptaacetyl methyl ester and a crystalline N-acetyl derivative. The former is a compound well suited for characterization purposes, and the latter makes available a model for comparison with the products of enzymatic degradation of hyaluronic acid. From quantitative paper chromatography, it appears that the testicular hyaluronidase digest of hyaluronic acid (from which hyalobiuronic acid is derived by mineral acid hydrolysis) is composed of a family of oligosaccharides of graded molecular weights, all higher than that of the N-acetyl derivative now prepared. The significance of the results of this investigation with respect to the structure of the disaccharide and the nature of the products of enzymatic degradation of hyaluronic acid will be discussed. (This work was supported by grants from the U. S. Public Health Service and from the Helen Hay Whitney Foundation.)

Assay for intrinsic factor in patients with pernicious anemia in remission given radioactive vitamin B_{12}. A. D. WELCH, V. SCHARF,* R. W. HEINLE AND G. C. MEACHAM.* *Depts. of Pharmacology and Medicine, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

Orally administered vitamin B_{12} produces little or no hematopoietic effect in patients with pernicious anemia unless intrinsic factor is administered simultaneously. The presence of materials with vitamin B_{12}-activity, presumably formed by bacteria in the intestine, has precluded the determination in the feces of unlabeled ingested vitamin. Studies with cyanocobalamin-Co^{58} (Merck & Co.), the gamma radiation of which can be measured in the presence of feces by scintillation counting, have shown that patients with pernicious anemia *in remission*, given oral doses of only 0.5 μg. (0.03 μc.) of the radioactive vitamin, excrete 70–95% of the cobalt in the feces. When gastric juice or concentrates of hog stomach are administered simultaneously with the vitamin, the amount of cobalt excreted is reduced to 5–30% of that ingested. A typical patient given 3 doses of radioactive vitamin B_{12} (0.5 μg.) orally, without intrinsic factor, eliminated 70, 95 and 95%, respectively, of the cobalt; concurrent administration of 10 mg. of a hog stomach fraction reduced the elimination to 25% and a greater effect was not produced by a dose of 100 mg. The findings in all patients suggest that intrinsic factor is involved in the removal of the vitamin from the gastrointestinal contents and form the basis of a technique for assay of intrinsic factor fractions. The methods are simple and relatively rapid as compared with the classical assay procedure requiring prolonged clinical and hemato-

logic observations in seriously ill, untreated patients.

Coagulation changes in isolated venous segments in the dog. STANFORD WESSLER, (introduced by BENJAMIN ALEXANDER). *Yamins Research Lab., Beth Israel Hosp., and Dept. of Medicine, Harvard Med. School, Boston, Mass.*

Isolated blood-filled venous segments were prepared in 35 dogs. In these isolated segments, containing 1–2 cc. of blood, grossly visible clots rarely appeared within 60 minutes but always formed within 120 minutes. In 6 dogs the prothrombin concentration of the blood in multiple isolated segments was determined at intervals ranging from 2 minutes to 9 hours after isolation of the segment. By the two-stage method prothrombin concentration remained unchanged for 4 hours despite evident deposition of a clot. Thereafter, prothrombin dropped slowly, but did not disappear completely in 9 hours. The slow rate of platelet disappearance and the one-stage prothrombin values, which paralleled the two-stage results, confirmed the marked deceleration of clotting afforded by endothelial tissue. It is evident that coagulation changes occur far more slowly in blood in contact with the endothelial lined surface of isolated venous segments than in other lined vessels currently employed in various *in vitro* technics. It should be emphasized that under the conditions of these experiments the blood is completely stagnant and the vascular segment entirely cut off from the circulation. The use of this approach may facilitate study of the earliest phases of the coagulation sequence.

Selective uptake of Ag^{111} by pure or mixed fibrous tumors. HAROLD D. WEST AND ALFONSO P. JOHNSON.* *Dept. of Biochemistry, Meharry Med. College, Nashville, Tenn.*

Radioative silver, a pure beta-emitter, administered as soluble nitrate has been shown to accumulate in infected areas (*Am. J. Roentgenol. & Rad. Therapy* 64: 831, 1950; *J. Lab. & Clin. Med.* 34: 1376, 1949). Further study has shown that selective Ag^{111} uptake is observed also in fibrous or mixed rat tumors. Five tenths ml. of a standard $Ag^{111}NO_3$ solution was injected into a hind leg muscle of albino rats with spontaneous tumors including 9 fibromas, 4 gastric carcinomas, 2 fibroadenomas and 6 albino rats with transplantable liver cell carcinomas. After 72 hours the animals were sacrificed, hearts, lungs, livers, kidneys, leg muscles and tumors were subjected to a wet ashing procedure (*Am. J. Roentgenol. & Rad. Therapy* 64: 831, 1950) and the silver isolated as chloride. One ml. samples of Ag^{111} solution carried through the ashing process simultaneously gave $Ag^{111}Cl$ to serve as a control. Specific activities averaged: hearts 15, lungs 54, livers 335, kidneys 68, muscle of the leg injected 1884, muscle of opposite leg 733 c/m for the whole tissue. Activities for fibromas averaged 7111, gastric carcinomas 257, fibroadenomas 7466, transplantable liver cell carcinoma 24. Average activity of control samples was 28994 c/m. Thus, uptake by fibromas and fibroadenomas was several hundred times higher than uptake of any normal tissue. It is concluded that use of Ag^{111} may provide a diagnostic method for detection of deep lying tumors of certain types and eventually, also a therapeutic method for internal irradiation of tumor cells.

Laboratory synthesis of some mercapturic acids and biological acetylation of the intermediate cysteines. HAROLD D. WEST AND GHARBHARAN R. MATHURA.* *Dept. of Biochemistry, Meharry Med. College, Nashville, Tenn.*

Certain problems related to the metabolism of diphenyl in the animal body made it desirable to undertake a study of the laboratory synthesis and biological acetylation of a number of aryl-substituted cysteins. Phenyl- (*J. Biol. Chem.* 151: 211, 1943), p-fluorophenyl- (*J. Biol. Chem.* 152: 599, 1944), p-bromophenyl- (*J. Biol. Chem.* 122: 333, 1937–38), benzyl- (*J. Biol. Chem.* 122: 133, 1937–38; 138: 369, 1941) and p-chlorophenyl-L-cysteine (*J. Biol. Chem.* 193: 133, 1951) have been shown to be acetylated in the animal body. Of the substituted benzene derivatives so far studied only the *para* isomer is known to undergo biological acetylation to give the corresponding mercapturic acid. Some of the *ortho-* and *meta-*substituted aryl-cysteines have now been synthesized in the laboratory and fed to albino rats (Wistar strain) to discover if the position of the substituent influences its acetylation *in vivo*. In addition the synthesis of some aryl-cysteines with substituents other than halogens in the benzene ring has been studied in a similar manner. The fate of these substances in the animal body, details of the synthetic work and physical constants of the new compounds will be presented. (This work was supported in part by research grant C-1524 from the National Cancer Institute.)

Azorubin-binding capacity of serum albumin of rats subjected to tourniquet shock and CCl_4-poisoning. ULRICH WESTPHAL, ROBERT DEARMOND, STANLEY G. PRIEST AND JOHN F. STETS (introduced by H. JENSEN). *Army Med. Research Lab., Fort Knox, Ky.*

A chromatographic method was used in determining the azorubin-binding capacity (ABC) of serum albumin. This capacity is considered characteristic of the ability of a protein to combine with anions. The free azorubin is separated

from that bound to albumin by adsorption on anionotropic aluminum oxide. Evidently the other serum proteins do not interfere with the combining of azorubin and albumin in this test. It was previously found that the ABC values of serum albumin are decreased in certain pathological cases (*Proc. Soc. Exper. Biol. & Med.* 76: 838, 1951). In the present studies, employing rats, the ABC values of serum albumin was lowered in animals subjected to tourniquet shock. Subnormal ABC values of the serum albumin were also observed in rats after treatment with carbon tetrachloride. In model experiments using crystalline human serum albumin (Cutter) it was found that addition of small amounts of anions (fatty and other acids) will decrease the ABC; 2.5 molecules of lauric acid/molecule albumin lowered the ABC by 45%; 1 molecule of acid/2 molecules of albumin still gave a definite lowering. It is suggested that a decreased ABC of serum albumin, observed under certain conditions, is caused by a firm binding of anions to the albumin and thus occupying the sites at which other anions as azorubin could combine. Results of dialysis experiments and electrophoretic mobilities are in accordance with this hypothesis.

Utilization of D-valine for growth by the rat. JULIUS WHITE, WILLIAM S. FONES* AND HERBERT A. SOBER. *Natl. Cancer Inst., Natl. Inst. of Health, U. S. Public Health Service, Federal Security Agency, Bethesda, Md.*

We have previously shown that D-valine is glycogenic in rats. Furthermore, α-ketoisovaleric acid is capable of supporting growth in rats on a valine free diet (*J. Biol. Chem.* 186: 641, 1950). These data indicate that D-valine may be available via inversion for protein synthesis and might therefore be able to support growth or maintain young rats. Previous reports (ROSE, *Physiol. Reviews* 18: 109, 1938) have indicated that D-valine does not support growth. Ten Osborne-Mendel rats weighing 110–112 gm. were fed a valine-free synthetic diet for 28 days, after which time the animals weighed 60–72 grams. Each animal then received 120 mg. of D-valine for 6 days. The average weight gain was 1 gm/day. Removal of D-valine from the diet resulted in an average loss of 1.2 gm/day. Addition of 120 mg. of L-valine daily resulted in an average weight increase of 1.9 gm/day. Two other valine-deficient rats (72 and 77 gm.) were given 120 mg. of D-valine by stomach tube (C^{13} atom % excess 9.65, $[\alpha]_D^{25} = -27.5$) for 3 days. The animals were sacrificed 72 hours later, livers removed, homogenized and dialized 18 hours against running tap water to remove free amino acids. The non-dializable fraction from each liver was hydrolyzed and valine isolated by ion exchange chromatography. The valine isolated had an $[\alpha]_D^{25} = +27°$ and 0.41 C^{13} atom per cent excess.

Action of insulin on oxidation of glucose and other foods. ARNE N. WICK AND DOUGLAS R. DRURY.* *Scripps Metabolic Clinic, La Jolla, and Univ. of Southern California, Los Angeles.*

If maximum insulin effect is induced in eviscerated animals the oxidation of glucose is greatly increased. At the same time, there is a reciprocal reduction in oxidation of other substances which were previously the source of energy. In this the result of specific inhibitions to particular metabolites brought about by insulin or is it an automatic adjustment resulting from the active acceleration of glucose oxidation? We have studied this question in eviscerated, nephrectomized rabbits by administering glucose and other fuels simultaneously to the animals while they were under maximum insulin action. The carbon of the glucose was labeled so that its rate of oxidation could be determined by following the activity of the CO_2 produced by the animal. We have found that beta-hydroxybutyrate will depress the rate of glucose oxidation induced by insulin. Lactate, acetate and propionate will also do this to a limited degree. We are studying the inverse of this finding by administering the other substances in the labeled form and determining whether or not the addition of insulin will depress their oxidation. This is being done with the aim of determining whether the suppression by insulin of the oxidation of non-glucose fuels is a general one or whether there are specific individual exceptions.

Manometric and spectrophotometric studies on cytochrome-*b*. CARL WIDMER, JR.* AND ELMER STOTZ. *Dept. of Biochemistry, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Oxidation of succinate using partially purified preparations of succinic dehydrogenase, cytochrome *b*, cytochrome oxidase and purified cytochrome *c* has been demonstrated using the Warburg apparatus. All components of the system except cytochrome *b* were in excess. No reaction occurred in the absence of cytochrome *b*. Cytochrome *b* has been found non-autoöxidizable. The amount of cytochrome *b* hematin was estimated spectrophotometrically ($\epsilon = 2.64 \times 10^4$ for the reduced band at 561 mμ). Under these conditions 1080 moles of cytochrome *b* iron were oxidized per minute at 3.4×10^{-7}M cytochrome *b* hematin (phosphate buffer, 0.05 M, pH 7.4). Increase in optical density at 550 mμ and 561 mμ was taken as evidence for the reduction of cytochrome *c* and cytochrome *b* respectively. Manometric experiments were made to parallel these whenever possible. DPN was shown incapable of

reducing cytochrome *b* either directly, through diaphorase, or through DPN-cytochrome *c* reductase. Xanthine dehydrogenase was found to catalyze the reduction of cytochrome *c* but not of cytochrome *b* by hypoxanthine. The napthoquinone, SN5949, (2 mg/liter) did not inhibit succinic dehydrogenase or the cytochrome oxidase system, but completely inhibited the succinoxidase system at a cytochrome *b* hematin concentration of 6.6 $\times 10^{-6}$ M. This compound blocked the reduction of cytochrome *c* by reduced cytochrome *b*. Antimycin, although not entirely specific in its action, appeared to have its greatest effect on succinic dehydrogenase. As little as 1.3 μg/ml. completely inhibits oxygen uptake by the reconstructed succinoxidase system.

Conversion of hypoxanthine to inosinic acid by pigeon liver enzymes. WILLIAM J. WILLIAMS* AND JOHN M. BUCHANAN. *Dept. of Physiological Chemistry, School of Medicine, Univ. of Pennsylvania, Philadelphia.*

Pigeon liver extract contains a soluble enzyme system which catalyzes the conversion of radioactive hypoxanthine to inosinic acid in the presence of ribose-5-phosphate and adenosine triphosphate (ATP). This system consists of at least two enzymes which may be separated by precipitating the protein between 0–15% ethanol (Fraction I) and 20–45% ethanol (Fraction II). Fraction II contains considerable nucleosidase activity and may be replaced in the enzyme system studied by the rat liver nucleoside phosphorylase preparation of Kalckar. We have no further evidence at present as to whether the nucleoside phosphorylase or some other enzyme in this unpurified fraction is responsible for the activity. Radioactive inosine is not converted to inosinic acid in significant quantities unless both Fraction I and 'nucleoside phosphorylase fraction' are present. Further, when incubated with these two fractions and ATP, radioactive hypoxanthine in the presence of unlabeled inosine is converted to radioactive inosinic acid to about twice the extent that radioactive inosine is converted to radioactive inosinic acid when unlabeled hypoxanthine is present. Radioactive inosine or radioactive hypoxanthine alone are both converted to inosinic acid to about the same extent in the presence of ribose-5-phosphate ATP, Fraction I, and 'nucleoside phosphorylase fraction.' These results indicate that inosine is not an intermediate in the conversion of hypoxanthine to inosinic acid, but that hypoxanthine and some derivative of a ribose phosphate ester react directly to give inosinic acid. The nucleoside phosphorylase fraction is involved in this reaction.

Structure of pepsin. MARTIN B. WILLIAMSON AND JOHN M. PASSMANN (introduced by HENRY H. BULL). *Dept. of Biochemistry, School of Medicine, Loyola Univ., Chicago, Ill.*

As part of a study on the structure of pepsin, the basic design of this enzyme has been characterized. Using Sanger's technique of labelling with 2,4-dinitrofluorobenzene, the terminal free amino group of pepsin was identified. The resulting dinitrophenyl (DNP) peptides and amino acid were not readily separable on buffered silica gel columns. However, the DNP-peptides and -amino acid could be identified by paper chromatography, with the proper solvent systems. An ether extract of hydrolyzed DNP-pepsin yielded 10 DNP-compounds. Nine of these were found to be DNP- peptides or -derivatives, while only one was identified as a DNP-amino acid. The R_f of this DNP-amino acid was identical with that of DNP-leucine and DNP-isoleucine. It was found that the terminal amino acid in pepsin is leucine. The elution of the DNP-compounds from the paper and the determination of the optical density of the eluate indicated that only one equivalent of terminal free amino group was present per mole of pepsin. Oxidation of the two cystine residues in pepsin and DNP-pepsin was carried out with performic acid. It was expected that more than one peptide might be identifiable, if the disulfide bonds serve as a bridge between individual peptide chains. Two peptides were identified by chromatography, in each case. It seems probable that the pepsin molecule consists of two peptides, one of which, a straight chain, has leucine at the terminal free amino end, the other being cyclic. (This work was supported by a grant from the Research Corp.)

Fractionation of formaldehydeogenic substances in urine extracts. HILDEGARD WILSON (introduced by ISIDOR GREENWALD). *Dept. of Chemistry, New York Univ. College of Medicine, New York City.*

The high and erratic titres of neutral formaldehydeogenic substances (NFS) in urines treated with β-glucuronidase (βG) suggested studies to test the assumption that these represent the true corticosteroid (CS) content. Urines were extracted at pH 5.0 with $CHCl_3$ to remove the free steroids (I), then re-extracted after treatment with βG (II), and finally at pH 1.0 in continuous ether extractors (III). Assays on each extract before and after benzene-water partition showed that II had much the highest titre, and that most NFS passed into the water extracts. These were therefore chromatographed on paper according to Burton *et al.* (*J. Biol. Chem.* 193: 769, 1951) and the $C_{21}O_3$, $C_{21}O_4$ and $C_{21}O_5$ strips eluted and assayed. Eluates of I and III gave titres comparable to the

material applied. Only a small fraction of the original high NFS of II was recovered, suggesting that much of it did not represent CS compounds. In an effort to separate CS glucuronides from this interfering NFS, urines at pH 1.0 were continuously extracted with ether. The extracted conjugates were treated with βG and re-extracted. Very litle NFS was found either in this fraction or among the free steroids. Treatment of the residual urine with βG yielded much more NFS both in crude fractions and eluates of papergrams. Although certain steroid glucuronides are known to be subject to continuous ether extraction, most βG hydrolyzable CS conjugates apparently were not removed. Detailed data will be presented, and their significance discussed.

Mechanism of hydrolysis by acetylcholinesterase. IRWIN B. WILSON (introduced by DAVID NACHMANSOHN), *Dept. of Neurology, College of Physicians and Surgeons, Columbia Univ., New York City.*

Previous studies have revealed the existence of two sites in the molecule of acetylcholinesterase: *1*) an anionic site which binds and orients cationic molecules; *2*) an esteratic site forming a covalent bond with the electrophillic carbon of the carbonyl group. A mechanism of hydrolysis has been proposed on the basis of these findings. Further studies have yielded more detailed information about the forces of interaction of small molecules, inhibitors and substrates, with acetylcholinesterase. The significance of the degree of alkylation of the amino group and the importance of the electrophillic character of the acyl carbon atom will be discussed. The relative importance of kinetic and equilibrium terms in the Michaelis-Menten constant and the effect of structure upon the inhibition by excess substrate will be considered.

Association of thyroxine with plasma proteins. RICHARD J. WINZLER AND S. R. NOTRICA.* *Dept. of Biochemistry and Nutrition, Univ. of Southern California and School of Medicine, Los Angeles.*

It has been established that the 'protein-bound iodine' of serum is largely thyroxine which can readily be extracted with butanol. It is not known, however, whether thyroxine is associated with specific proteins, although some evidence exists that the PBI is primarily associated with a protein of the albumin fraction. This problem has been approached using zonal electrophoreis on filter paper to separate the plasma proteins of hyperthyroid patients given therapeutic amounts of radioactive iodide. One tenth milliliter samples of serum from patients given 7–20 millicuries of I^{131} 24–72 hours previously were subjected to paper electrophoresis. In addition to small amounts of radioiodide which migrated faster than the plasma proteins, radioactivity was largely confined to two areas, a major one somewhat slower than albumin and a minor one with the mobility of alpha-2-globulin. The radioactivity was readily extracted from the paper with butyl alcohol and was shown, by paper chromatography, to be thyroxine. Euthyroid patients given radioactive iodide for heart conditions showed a similar distribution of radioactivity, but at a much reduced level. Two thyroidectomized patients given 100 millicuries of I^{131} in treatment of metastatic thyroid cancer had appreciable protein-bound radioactivity which was associated entirely with the alpha-2-globulin peak. The results of these experiments suggest that plasma thyroxine is loosely associated with one or perhaps two specific proteins, the major one having, on filter paper, an electrophoretic mobility slightly less than albumin.

Basal metabolic changes due to surgical removal of aortic paraganglia in the guinea pig. OZRO B. WISWELL (introduced by ERNEST BEERSTECHER). *Dept. of Anatomy, Univ. of Texas, Dental Branch, Houston.*

Thirty guinea pigs of each sex were subjected to basal metabolic evaluation to determine the average normal basal metabolic rate. Twenty animals of each sex were subjected to operative procedures under ether anesthesia during which the aortic paraganglia were extirpated. In the male operative group there was a postoperative BMR increase for 15 days reaching 15% above the preoperative average normal male BMR. The group average normal BMR was reached again on the 28th day but continued downward until the experiment was terminated on the 90th postoperative day at which time the BMR was evaluated as being 19% below the average preoperative male group normal BMR. The female group of guinea pigs reached their highest postoperative level of 2.5% above the preoperative average group normal BMR on the 10th day and the decline was then so rapid that the preoperative female average was reached by the 16th postoperative day. Continuing the BMR evaluation for the period of the experiment a final point of 13% below the average normal BMR for the group was reached. Unoperated and mock operated controls were continually evaluated. Animals subjected to this extirpative procedure changed their oxygen intake in relation to their body weight. The data indicate that aortic paraganglia are important physiologically due to the apparent change in the BMR upon extirpation.

Mechanism of action of dinitrophenol. ROBERT F.

WITTER AND ETHEL NEWCOMB.* *Dept. of Biochemistry, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

In order to investigate the theory that dinitrophenol is an activator of phosphatases, the effects of dinitrophenol on the rates of hydrolysis of various phosphate compounds by particulate fractions of rat liver were determined by measurement of the rates of liberation of orthophosphate under anaerobic conditions. Dinitrophenol increased the rates of hydrolysis of ATP, ADP and pyrophosphate in fresh washed particles but not in frozen or acetone-dried particles or fresh mitochondria prepared in hypertonic sucrose-isotonic potassium chloride, indicating that dinitrophenol may activate 'phosphatases' present in fresh washed particles. In both washed particles and mitochondria, dinitrophenol prevented the oxidation of fatty acids and the maintenance of the ATP level by malate oxidation without influencing the rate of oxidation of malate. In the presence of dinitrophenol, aerobic conditions did not affect the hydrolysis of ATP but inhibited that of pyrophosphate. Dinitrophenol had no effect on the pyrophatases of rat erythrocytes and yeast or on the phosphocreatine or phosphopyruvate transphosphorylases of rabbit muscle. Studies of the effects of incubation with dinitrophenol on the ability of washed particles to oxidize sorbate showed that dinitrophenol did not cause an irreversible change in the fatty acid oxidase. Dinitrophenol inhibition of sorbate oxidation is not due to the low ATP supply since maintenance of the ATP level with the phosphocreatine system did not overcome dinitrophenol inhibition. Previous work from this laboratory indicated that acetoacetate formation from pyruvate unlike that from sorbate was not inhibited by dinitrophenol. Thus dinitrophenol may inhibit fatty acid oxidation at some stage prior to acetoacetate formation.

Roles of magnesium chloride and pH in preservation of fatty acid oxidase activity of mitochondria heated at 30°. ROBERT F. WITTER AND ETHEL NEWCOMB.* *Dept. of Biochemistry, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Some factor or factors present in the reaction mixture used to study fatty acid oxidation at 30° by rat liver mitochondria appear to prevent a loss in fatty acid oxidase activity which occurs when mitochondria are heated at 30° for 5 minutes in the absence of the reaction mixture. In an effort to discover these factors, mitochondria were incubated at 30° for 5 minutes in the presence of various components of the reaction mixture, and the subsequent loss in fatty acid oxidase activity was determined. Included in the reaction mixture were buffer at pH 8.0, potassium chloride, cytochrome *c*, magnesium chloride, ATP, phosphate, the model substrate sorbate, and its necessary activators α-ketoglutarate or malate. Incubation of the mitochondria in potassium chloride (pH 6.5–6.7) inhibited the oxidation of sorbate, malate, and α-ketoglutarate. Incubation at pH 8.0, the pH of the reaction mixture, inhibited only the oxidation of sorbate. Incubation at pH 8.0 with phosphate completely inhibited the oxidation of sorbate, malate, and α-ketoglutarate. The addition of magnesium chloride before incubation prevented the inhibitory effects of incubation at pH 8.0 in the presence or absence of phosphate. Magnesium chloride plus ATP but not ATP alone was sometimes more effective than magnesium chloride alone in the preservation of the ability to oxidize sorbate. These results indicate that pH and the presence of magnesium chloride may play important roles in the stabilization of the fatty acid oxidase.

Growth of *Hemophilus pertussis* from ultrasonic extracts of L forms. RUTH G. WITTLER* AND LOUIS PILLEMER. *Inst. of Pathology, Western Reserve Univ., Cleveland, Ohio.*

A smooth strain of *H. pertussis*, grown in modified Cohen-Wheeler broth containing 1.5% glycine, transforms to the L form after 24 hours of incubation. Such cultures were subjected to ultrasonic vibrations which disintegrated the formed elements. The clear ultrasonic extracts were fractionated by high speed centrifugation and the final supernatants showed no visible forms by light microscopy. Nevertheless, under carefully controlled conditions, true bacillary cultures of *H. pertussis* have consistently grown out on Bordet-Gengou media from these supernatants. It would appear from the evidence at hand that the bacteria were produced from L forms resistant to ultrasonic treatment or from 'submicroscopic particles.'

Fermentation of glucose-3,4-C^{14} and glucose-1-C^{14} by Propionibacterium arabinosum. HARLAND G. WOOD AND FREDRICK LEAVER.* *Dept. of Biochemistry, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

It usually has been considered that the propionic acid fermentation of glucose occurs by cleavage to two C_3-compounds and that succinate is formed either by CO_2 fixation or by a condensation in a Thunberg or Krebs cycle, and that priopionate is formed by direct reduction of a C_3-intermediate or by decarboxylation of succinate. Accordingly glucose-3,4-C^{14} would yield carboxyl labeled acids and glucose-1-C^{14} unlabeled CO_2. From glucose-3,4-C^{14} the products were labeled as follows: CH_3(7.5) - CH_2(9.2) - COOH(63.4), COOH(47.0) - CH_2(17.2) - CH_2 - COOH, CH_3(7.4) - COOH(5.8),

CO_2(55) and from glucose - 1 - C^{14}: CH_3(14.3) - CH_2(15.7) - COOH(8.1), COOH(4.5) - CH_2(15.5) - CH_2 - COOH, CH_3(15.1) - COOH(5.3), CO_2(19.8). The values in parentheses are specific activities in percentage of the specific activities of the labeled positions of the substrate. Thus contrary to expectations, approximately 8 and 17% of the α and β positions of the propionate and succinate respectively came from the 3,4-carbons of glucose and about 20% of the CO_2 was from the 1-position. In neither fermentation did the propionate have the same tracer pattern as the succinate as would be expected if propionate were formed entirely from succinate. Gibbs and DeMoss (*Federation Proc.* 10: 189, 1951) with *Leuconostoc meserenteroides* and labeled glucose showed the following occurs: $C^* - C - C^\circ - C^\circ - C - C \rightarrow C^*O_2 + CH_3 \cdot C^\circ H_2OH + C^\circ OOH \cdot CHOH \cdot CH_3$. Our results differ in that the 1- and 3,4-positions entered every position of each product. One possible explanation is that there is a C_3 and C_3 split to yield in part a symmetrical C_3, e.g. dihydroxyacetone. The randomization in the C_3 plus that in a C_4-dicarboxylic acid together with a C_1 and C_5 type split could account for the distribution of tracer.

Thiocyanate formation from cystine derivatives. JOHN L. WOOD AND SHIRLEY LUCILLE COOLEY.* *Univ. of Tennessee, Memphis.*

The spontaneous, non-enzymatic formation of thiocyanate ion from some possible precursors has been investigated. The reaction product of cystine and cyanide ion, α-amino-β-thiocyano propionic acid, released no thiocyanate ion on standing in solution at 37°. Either acetylation or esterification of α-amino-β-thiocyano propionic acid produce a labilization of the thiocyano group so that on incubation thiocyanate ions were liberated slowly. The N-acetyl ester was much more labile. The reaction product of cyanide and oxidized glutathione produced thiocyanate ion very readily. A protein such as egg albumin when incubated with cyanide solution released thiocyanate ion. Plasma also produced thiocyanate ion on addition of cyanide. The results indicate that cystine, when in peptide combination, reacts with cyanide to produce thiocyanate ion.

Amino acid composition and nitrogen metabolism of *Tetrahymena geleii*. CHUNG WU AND JAMES F. HOGG (introduced by A. A. CHRISTMAN). *Dept. of Biological Chemistry, Univ. of Michigan, Ann Arbor.*

Two strains of *T. geleii* E, one (P) maintained in a peptone medium and the other (S) maintained in a synthetic medium, were studied. Strain P was grown in peptone and in synthetic media to obtain cell preparations P-P and P-S; strain S in the synthetic media yielded preparation S-S. The cell preparations and filtrates of the synthetic medium were analyzed for 18 amino acids (microbiological assay), nucleic acid, ammonia, creatinine, urea, uric acid and ergothioneine. The latter 4 were not found in significant quantity. Total amino acid N plus nucleic acid N equalled 85% of total N for all preparations but S-S had twice the nucleic acid content of P-S. The amino acid patterns of the cell preparations showed definite changes for adaptation to the synthetic medium. There was also a net increase of acidic components (dicarboxylic amino acids and nucleic acid) in S-S. The analyses of the cells and culture filtrates completely accounted for the total N of the original medium. Thus the only significant excretory compounds of N present in the filtrates were ammonia, glycine, glutamic acid, alanine, aspartic acid and tyrosine. A comparison of the minimal growth requirements, total utilization and the cellular incorporation of the 11 essential amino acids demonstrated that a relatively large catabolism of arginine, serine, threonine and tryptophan was essential to growth. The total utilization of histidine was experimentally insignificant but the growth requirement far exceeds the cellular incorporation found. For the other amino acids, minimal growth requirements approximated the cellular incorporations.

Determination of hippuric acid in normal urine—a study in differential extraction. HSIEN WU AND DAISY YEN WU.* *Dept. Biochemistry Med. College and School of Dentistry, Univ. of Alabama, Birmingham.*

Ether extracts hippuric acid (HA) easily from urine, but it extracts also some urea and other N compounds (X). If HA is to be determined by Kjeldahl method, it is necessary to separate it from urea and X. If uniform droplets of ether are allowed to pass through an aqueous solution of HA, the amount of HA extracted bears a quantitative relation to the volume of ether. A knowledge of this relation can be utilized for the determination and identification of HA. With 10 ml. normal urine and 1 ml. sat. $KHSO_4$ in an extraction tube, 400 ml. of ether will extract all the HA but only 1/170 of urea and 1/3 of X. If the ether is distilled off, the residue dissolved in water and re-extracted with 400 ml. ether, HA is again all extracted while the amounts of urea and X are reduced by the same fractions as before. Five or 6 re-extractions with a single tube will eliminate practically all the urea and X. In an alternative and more convenient procedure, the ether after passing through the tube containing urine is allowed to pass through a series of 3 tubes each charged with 10 ml. water and 1 ml. $KHSO_4$. With this arrangement, 600 ml. ether will extract all the HA but very small fractions of urea and X, and one or two re-extractions will practically eliminate all the urea and X. HA extracted from

urine and purified as described above can be identified by its extraction curve.

Enzymatic synthesis of glutathione. S. YANARI*, J. SNOKE* AND K. BLOCH. *Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.*

Freshly prepared extracts of acetone dried pigeon liver synthesize glutathione from the 3 constituent amino acids or from glutamyl cysteine and glycine with the sole addition of ATP as an energy source. When such extracts are aged, activity is greatly reduced. Restoration of this activity requires the addition of both ATP and either glycogen, hexosediphosphate, phosphoglycerate or phosphoenolpyruvate. That the energy requirements of this system are met by glycolytic reactions which generate high-energy phosphate but not by additional ATP is attributable in part to ATPase. These findings provide an explanation for the earlier claim (BLOCH, *Federation Proc.* 10: 163, 1951), now to be revised, that glutathione synthesis requires a heat stable co-factor. Extracts from various mammalian acetone dried livers, previously found to be inactive, synthesize glutathione at the same rate as pigeon liver extracts provided phosphoglyceric acid is added with ATP. Unlike acetone dried pigeon liver, mammalian livers lack components necessary for adequate glycolysis. The enzyme system which catalyzes the synthesis of glutathione from glutamylcysteine and glycine has been purified about 100-fold by a combination of acid precipitation, ammonium sulfate and ethanol fractionation. Such preparations contain considerably less ATPase activity than the crude extracts and show only a slight increase in activity upon addition of phosphoglyceric acid and the enzymes necessary to generate high-energy phosphate. Purified preparations do not synthesize glutathione from the 3 amino acids. Recombination of various fractions obtained during purification failed to reveal the presence of more than one enzyme involved in the synthesis of glutathione from glutamylcysteine and glycine.

Poly-ribophosphate, an immunologically active type-specific substance. STEPHEN ZAMENHOF, GRACE LEIDY,* PATRICIA L. FITZGERALD,* HATTIE E. ALEXANDER* AND ERWIN CHARGAFF. *Dept. of Biochemistry and Pediatrics, Columbia Univ., New York.*

Experiments on the purification by electrophoresis of the transforming principle of *Hemophilus influenzae* type b showed the type-specific immunologically active substance not to be separable from pentose nucleic acid (PNA). The purified substance was known to be orcinol-positive and to contain phosphorus, but little nitrogen (MACPHERSON *et al., J. Immunol.* 52: 207, 1946). Comparable preparations have now been found to exhibit no U.V. absorption. Chromatography revealed ribose as the only sugar. The presence of equimolar amounts of organic phosphorus and ribose and the absence of secondary phosphoryl groups show this substance to be a poly-ribophosphate (PRP) in which phosphoric acid serves as connecting link. PRP is non-reducing; mild alkaline or acid hydrolysis produced dialysable fragments which were still non-reducing, but contained 0.9 mole secondary phosphate. Intestinal phosphatase dephosphorylated these fragments almost completely, leaving a ribose-containing, non-reducing residue. The sugar, therefore, did not carry phosphoric acid on carbon 1. The rates of liberation of inorganic phosphate by acid hydrolysis and of color production in the orcinol reaction were identical with those exhibited by yeast adenylic acid. The course of production of reducing sugars by acids suggested the presence of glycosidic linkages. Crystalline ribonuclease breaks PRP and destroys its immunological activity. A plausible formulation of PRP could regard it as consisting of a poly-ribophosphate chain, as it exists in PNA, in which the place of the purines and pyrimidines is taken by a second similar chain, linked to the first in 1-1′ glycosidic linkages.

Spleen DPNase and mechanism of inhibition by nicotinamide. LEONARD J. ZATMAN (introduced by SIDNEY P. COLOWICK). *McCollum-Pratt Inst., Johns Hopkins Univ., Baltimore, Md.*

Among the tissues examined (ZATMAN AND QUASTEL, in preparation), spleen appears to be one of the richest sources of the DPNase which attacks the nicotinamide-ribose bond (approx. 10 times as active as brain). Differential centrifugation of a spleen homogenate and washing with 0.02M bicarbonate resulted in a 6 times purification on a protein basis; attempts to solubilize the enzyme were, however, unsuccessful. This washed particle preparation split 33 μmoles DPN/mg. protein/hour, and was inhibited 50% by 1.5×10^{-3}M nicotinamide. Desamino-DPN was split at 25% the rate for DPN, and this reaction also was nicotinamide sensitive. Nicotinamide mononucleotide was not attacked by the preparation whilst the riboside was split at about one-fifth the rate for DPN in the phosphate buffered medium; in the absence of phosphate, however, the riboside was not attacked, indicating the presence of a phosphorylase of the type described by Rowen and Kornberg (*J. Biol. Chem.*, 193: 497, 1951). The splitting of DPN does not depend on the presence of phosphate. On removal of the nicotinamide from an inhibited system by dialysis, full enzyme activity is restored. In presence of a concentration of nicotinamide giving 50% inhibition of the enzyme, increasing the DPN concentration 27-fold had no significant effect;

this result indicates that the inhibition by nicotinamide is of a non-competitive type. Further experiments using radioactive nicotinamide have been carried out, and the results will be discussed with respect to the mechanism of nicotinamide inhibition.

Action of cortisone acetate on hemolysis produced by the enzymic formation of lysolecithin from dimyristoyl lecithin. E. A. ZELLER. *Dept. of of Biochemistry, Northwestern Univ. Med. School, Chicago, Ill.*

The synthesis of phospholipids of proven configuration and constitution by E. BAER and associates makes it possible to study the factors that decide which of the two fatty acids will be liberated from phospholipids by the action of phospholipase A (for nomenclature see: J. B. SUMNER AND K. MYRBÄCK, *The Enzymes*, I/986 1951). *L*-a-dimyristoyl lecithin was incubated at 37° with snake venom and in the presence of phosphate buffer pH 7.2, 0.5 mM. $CaCl_2$, isotonic NaCl solution, and washed human erythrocytes. The process was stopped by ice-cold NaCl solution. The remaining unhemolyzed cells spun down and the hemoglobin in the supernatant was measured spectrophotometrically. Erythrocytes, corresponding to 0.05 ml. of blood, are hemolyzed within 15 minutes by 2 μg. of *Naia naia* venom; *Vipera aspis* venom has nearly the same activity. This process shows the characteristics of phospholipase A. Therefore, a fatty acid need not be unsaturated in order to be split from lecithin by this enzyme. The position, α or β, is more likely the decisive factor. When as little as 0.05 millimolar and less cortisone is added to the system, hemolysis is markedly inhibited. Higher concentrations of cortisone are less effective. This result may be related to the enhancing effect of cortisone on hemolysis caused by cobra venom in the absence of lecithin. Other forms of hemolysis are also affected by cortisone. These results can be explained by the assumption that this steroid is able to fit into strategic positions of the lipoid layer of the cell membrane.

Action of basic antibiotics on bacterial diamine oxidase and guanidine deamidinase. E. A. ZELLER, L. S. VAN ORDEN,* AND W. F. KIRCHHEIMER.* *Depts. of Biochemistry and Bacteriology, Northwestern Univ. Med. School, Chicago, Ill.*

Two enzymes of the amine metabolism are influenced by streptomycin: *1*) the recently described (*Federation Proc.* 10: 273, 1951) guanidine deamidinase (GD) which liberates urea from arcaine. Other guanidines attacked by *Mycobacterium smegmatis* are agmatine and L-arginine. *2*) Diamine oxydase (DO). Since many other antibiotics are bases it was investigated whether the latter attack the same enzymes. The methods used were the same as previously described. To the suspension of *M. smegmatis* putrescine was added as substrate for DO, agmatine for GD. 0.01 mM. viomycin, 0.01 mM. neomycin, and 0.1 mM. hydroxyl-streptomycin almost completely inhibit DO from the beginning of the reaction as indicated by the oxygen uptake and the ammonia formation. DO of a streptomycin resistant strain is nearly as much affected as DO of a streptomycin sensitive strain by viomycin and neomycin while DO of the resistant strain is not inhibited by hydroxylstreptomycin. 0.01 mM. viomycin completely blocks GD while the other antibiotics, in the above concentrations, are somewhat less effective. Comparing GD of streptomycin sensitive and resistant strains similar results as with DO were obtained. The behavior of the two enzymes toward the antibiotics follows closely the reaction of the whole microorganisms. One group, including streptomycin, dihydrostreptomycin, and hydroxylstreptomycin, does not act on DO of streptomycin resistant strains, while the other group, including streptothricin, chloroamphenicol, viomycin, pyocyanine, and neomycin, does. It takes 4–8 hours to determine to which group a new antibiotic belongs.

Purification of human red cell acetylcholinesterase. CHARLES A. ZITTLE, EDWARD S. DELLA-MONICA* AND JONATHAN H. CUSTER.* *Eastern Regional Research Lab., Philadelphia, Pa.*

Three hundred-fold purification of the acetylcholinesterase in human red cells has been obtained by the following procedure. Red cell paste is lysed with 6 volumes of water and the pH lowered to 6.0 with acetic acid. The cell membranes, which clump at this pH, are permitted to settle and are washed 7 times, each time with 13 volumes of 0.01 M monosodium phosphate. The membranes are finally collected in a compact form by centrifuging and suspended in ethanolamine buffer pH 9.6. This colloidal solution contains almost all of the original esterase activity. To this solution is added Tween 20 (5%), ammonium sulfate (1.03 M), dipotassium phosphate (0.1 M) and toluene (1.7%). After emulsification by shaking, the mixture is filtered; about 50% of the esterase is obtained in soluble form in the filtrate. The preparation is freed of salts and is treated with Lloyd's reagent at pH 5.0 and subsequently with $Ca_3(PO_4)_2$ at pH 7.0. Large amounts of non-esterase proteins are removed by this procedure. This preparation at a 1% protein concentration is clear, still contains some hemoglobin and is 300 times more active per unit of protein than the starting material. In electrophoresis on filter paper at pH 8.4 the esterase moves toward the positive electrode; it has about the same mobility as hemoglobin.

AMERICAN SOCIETY FOR PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

Forty-second Annual Meeting

NEW YORK CITY, APRIL 14–18, 1952

(An asterisk following an author's name indicates "by invitation")*

Nervous system effects of 4-alkoxy-β-(1-piperidyl) propiophenones. BENEDICT E. ABREU, ALICE B. RICHARDS* AND GEORGE R. BURCH.* *Research Dept., Pitman-Moore Co., Indianapolis, Ind.*

Depression of the tremor in the canine tremor syndrome has been previously reported for a series of alkoxy benzilates (*Federation Proc.* 10: 275, 1951). That and other studies demonstrated that peripheral atropine action is practically absent, but that signs of central atropine effects are constantly present. Because cardiac arrhythmias and block occurred frequently in the therapeutic dose range, with the benzilates, these agents were discarded as potentially useful antitremor drugs. The present series includes a group of 4-alkoxy-β-(1-piperidyl) propiophenones with from 2–8 carbons either branched or in a straight chain. (See RICHARDS, A. B. *et al.*, for general pharmacology). A maximum in tremor reducing and/or abolishing potency is reached in the 4 and 5 carbon substitutions. (See BUNDE, C. A. *et al.*, motion picture, for effects of the *n*-butoxy derivative on the canine tremor syndrome.) Evaluation of the mechanism of this action in portions of the canine nervous system reveals that none of these agents exert significant skeletal neuro-muscular depressant effects, but all diminish the tibial nerve-tibialis anterior muscle flexion reflex with maximum activity appearing as in the tremor syndrome dogs with the 4–5 carbon atom substitutions. This flexion reflex depression as with that produced by mephenesin is antagonized by strychnine.

Intravenous vs. intra-aortic epinephrine. RAYMOND P. AHLQUIST. *Med. College of Georgia, Augusta.*

Intravenous epinephrine produces a greater pressor response than does the same dose administered intra-aortically, in anesthetized, atropinized dogs. Intra-aortic injections of epinephrine made through a small, radiopaque catheter positioned by fluoroscopic observation produced the following comparative pressor responses (intravenous = 100): aortic arch 90, thoracic aorta 60, diaphragmatic aorta 30, renal aorta 60, and aortic bifurcation 40. To determine the distribution pattern of the injected epinephrine the following were recorded: the aortic pressure pulse contour (for the myocardial action), intestinal activity, renal urine flow, and femoral or carotid blood flow. The results indicate that the magnitude and character of the pressor response are determined by the proportional amounts of the injected dose delivered to the various vascular beds on the first circulation. The characteristic type of response of each vascular bed, the time of occurrence and the degree of myocardial stimulation, and the proportion of the dose first circulated through the liver are apparently the principal factors which determine the epinephrine pressor response.

Some pharmacologic effects of mono-halogenated tetrahydrothiophene 1,1-dioxides (sulfolanes). WILLIAM M. ALEXANDER,* BENEDICT E. ABREU AND H. ELDRIDGE FAITH.* *Research Dept., Pitman-Moore Co., Indianapolis, Ind.*

Three derivatives of tetrahydrothiophene-1,1-dioxide in which the substitutions were made in the 3 position were found to be respiratory stimulants. Intravenous LD_{50}s for white mice in mg/kg. were as follows: iodo- 9.3, bromo- 25 and chloro- 75. In both mice and dogs, the compounds are central nervous system stimulants with a toxicity pattern resembling that of pentylenetetrazol. Employing 4 dogs at each dose level, the drugs showed the same order of toxicity as was found for the mice. Doses up to 25 mg/kg. for the bromo- and the chloro- compounds and up to 5 mg./kg. for the iodo-derivative did not alter cardiac rate of dogs anesthetized by pentobarbital sodium by more than 10% of the control. Blood pressure was only slightly depressed at these dose levels, and intestinal activity was not significantly affected by any of the drugs. Both rate and stroke volume of respiration were markedly increased by the above doses of the compounds, falling to the control level in about 20 minutes. The 3 drugs and pentylenetetrazol were compared on a molar basis for respiratory stimulating potency in anesthetized dogs. Using increased minute volume as the criterion of potency, the compounds may be arranged in the following order of increasing activity: pentylenetetrazol, the chloro-, the bromo-, and the iodo- sulfolanes.

Effect of drugs on the oxygen consumption of virus infected tissue cultures. JULIAN L. AMBRUS, CLARA M. AMBRUS, CATHERINA N. SIDERI AND J. W. E. HARRISSON (introduced by I. T. BECK). *Dept. of Pharmacology and Biochemistry, Philadelphia College of Pharmacy and Science, Philadelphia, Pa.*

During chemotherapeutic experiments, it was observed that certain compounds which decreased virus growth in infected tissues may interfere with metabolic processes of the host cells. A method has been designed whereby cultures of chick embryo tissues and isolated extraembryonic membranes can be maintained in the Warburg apparatus for several days. By using a suitable shaking device, such cultures may be employed similarly to roller tube cultures for the propagation of viruses. Several species of the latter were grown satisfactorily in this way. Oxygen consumption was registered continuously; carbon dioxide production, only at the end of the experimental period. Experiments with vaccinia virus will be reported. Multiplication of the virus was determined by titration in susceptible rabbits. Neurotropic vaccinia virus (strain WR) did not affect the oxygen consumption of the host cells. Thyroglobulin increased metabolism of the tissues, but did not alter multiplication rate of the virus. Some other substances which are claimed to increase oxygen consumption of isolated tissues are presently under study. The effect of compounds which decreased multiplication of the virus could usually be related to the toxic effect on the host tissue. The use of this method in chemotherapeutic studies will be discussed briefly.

Effect of adrenocorticotrophic hormone on blood glucose and insulin hypoglycemia. SAUL B. APPEL,* JOSEPH L. GLUCK,* STANLEY REICHMAN,* ALFRED MILLER,* ABRAHAM GOLDMAN,* CHARLOTTE SPRINGER,* AUSTIN A. SCHLECKER,* MILTON B. ROSENBLUTH* AND HERBERT S. KUPPERMAN. *Third (New York Univ.) Med. Div., Goldwater Memorial Hosp., and Dept. of Therapeutics, New York Univ.-Bellevue Med. Center, New York City.*

The influence of intravenous ACTH infusion upon fasting blood glucose and hypoglycemic response to intravenous insulin was determined in patients demonstrating normal glucose and insulin tolerance. Eosinophil counts and urinary glucose were also followed. ACTH produced a significant elevation in the fasting blood glucose. Depth of insulin hypoglycemia was significantly diminished by adequate doses of ACTH when infused for 4 hours prior to the injection of insulin, but the duration of insulin hypoglycemia was not significantly influenced. It would appear that the dosage and duration of ACTH infusion can be correlated with the magnitude of these effects. An attempt was made to construct dosage-response curves utilizing the effect of induction of hyperglycemia and the inhibition of insulin hypoglycemia by ACTH. The possible use of such curves for human bio-assay will be discussed.

Failure of excess vitamin B_{12} in influencing metabolic and toxic effects of thyroid U.S.P. XIV. JOHN DALE ARCHER,* JOE B. NASH,* ROBERT G. BROWN* AND GEORGE A. EMERSON. *Univ. of Texas Med. Branch, Galveston.*

Effects of vitamin B_{12} on viability and growth of thyroid- or thyroxin-treated rats deficient in this vitamin have been noted by several authors; none describes actions of vitamin B_{12} in modifying metabolic effects of large doses of thyroid substance in rats on a relatively adequate diet. Forty-five young female rats were divided into 3 groups of *ca.* the same mean body weights, within 3%, and maintained on Purina dog chow. Thyroid was given intragastrically for 16 days to 2 groups, as a suspension of the finely powdered drug, in a dose of 450 mg/kg.; one of these groups also received 16 daily doses of 75 μg/kg. of vitamin B_{12}, intraperitoneally. O_2 consumption, estimated manometrically, and body weights were noted during the last 8 days of treatment. No significant differences in either metabolic rate or weight were noted between the 2 groups receiving thyroid, but there was a substantially increased metabolic rate and a failure of growth in both treated groups as compared with the untreated controls. Similar results were found with thyroxin.

Action of veratramine and of sympathomimetic amines upon the automaticity of the atrioventricular node. R. B. ARORA,* E. MEILMAN* AND O. KRAYER. *Dept. of Pharmacology, Harvard Med. School, Boston, Mass.*

In dogs in Dial-urethane anesthesia atrioventricular (A-V) nodal rhythm was established by clamping off the area of the sino-auricular node. The electrocardiogram was recorded with a Grass ink-writing oscillograph. Heart rate was counted and the rhythm ascertained from the electrocardiographic records. Conventional bipolar lead II and unipolar pericardial leads were used. Veratramine in doses of 0.01–0.1 mg/kg. given intravenously decreased the rate of impulse generation in the A-V node; temporary cessation of the heart beat occurred with the highest doses. The effect wore off gradually similar to the action of veratramine upon the sino-auricular node (KRAYER, O. *J. Pharmacol. & Exper. Therap.* 96: 422, 1949). Sympathomimetic amines (L-epinephrine, L-nor-epinephrine, isuprel, tuamine) increased the A-V nodal activity. Very often, however, an ectopic pacemaker in the remaining atrial tissue was activated by the sympatho-

mimetic amines. The increase in A-V nodal activity caused by the sympathomimetic amines (as well as the increased activity of the ectopic atrial pacemaker) were antagonized by veratramine. Atropine (1 mg/kg.) did not prevent or abolish the action of veratramine upon A-V nodal activity.

Micromethod for the colorimetric determination of tetracaine. M. E. AUERBACH, DAVID L. DAVIS AND FRANCIS F. FOLDES (introduced by M. L. TAINTER). *Sterling-Winthrop Research Inst., Rensselaer, N. Y. and Dept. of Anesthesia, Mercy Hosp., Pittsburgh, Pa.*

A method utilizing bromothymol blue for the colorimetric determination of quaternary ammonium salts was published by Auerbach (*Anal. Edit. Eng. Chem.* 15: 492, 1943). This method was adapted to the determination of tetracaine. If the tetracaine is to be determined in plasma or spinal fluid the proteins are precipitated with 5% $HgCl_2$. With protein-free tetracaine solutions this step can be omitted. The protein-free tetracaine solution is buffered to pH 6 with a sodium acetate-acetic acid buffer. Following the addition of a dilute alkaline bromothymol blue solution the system is shaken out with a 10:90 ethylene chloride-toluene mixture. The bromothymol blue forms a yellow complex salt with tetracaine. This complex salt is soluble in, and is quantitatively extracted by the ethylene chloride-toluene mixture. The free dye is practically insoluble in this solvent and remains in the water phase. After the solvent layer is separated from the water phase by centrifugation the optical density of the tetracaine-dye is determined at a wave length of 410 mμ in a photoelectric colorimeter. The relationship between the tetracaine concentration and optical density follows the Beer-Lambert law. If the solvent layer containing the yellow tetracaine-dye salt goes over into the NaOH layer where a blue color develops. The maximal optical density of this color is 615 mμ. This reaction also follows the Beer-Lambert law. With identical tetracaine concentrations the optical density of the blue dye is about twice as high as that of the yellow tetracaine-dye salt. Samples containing 5–40 μg. of tetracaine can be determined with satisfactory accuracy. The presence of the hydrolysis products of tetracaine do not have any significant effect on the color intensity. The method described was used successfully for the determination of tetracaine in water, human plasma, and spinal fluid.

Effect of pre-incubation on the inhibitory effect of neostigmine and dimethylcarbamate of (2-hydroxy-5-phenylbenzyl) trimethylammonium bromide (RO2-683) on the enzymatic hydrolysis of procaine. MANUEL AVEN, DAVID L. DAVIS AND FRANCIS F. FOLDES (introduced by L. O. RANDALL). *Dept. of Anesthesia, Mercy Hosp., and Section on Anesthesiology, Dept. of Surgery, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.*

It was reported by Kisch (*Exp. M. Surg.* 1: 84, 1943) that pre-incubation of neostigmine with serum increased its inhibitory effect on procaine esterase. We also found that the temperature and the duration of preincubation greatly influenced the inhibitory effect of various stigmine-type compounds on the enzymatic hydrolysis of procaine in plasma. A detailed study of this problem was undertaken with 2 of these inhibitors (neostigmine and RO2-683). Several plasma samples containing neostigmine or RO2-683 in 1:1,000,000, 1:5,000,000 and 1:20,000,000 dilutions were pre-incubated for various lengths of time. At the end of the pre-incubation period 0.1 μg/ml. of procaine was added and the incubation continued for 10 minutes. To 1 sample in each series no inhibitor was added, and to another the inhibitor and procaine were added simultaneously. The inhibitory effect observed was expressed as:

$$\% \text{ inhibition} = 100 - \frac{\text{procaine hydrolyzed with inhibitor}}{\text{procaine hydrolyzed without inhibitor}} \times 100$$

It was found that when the inhibitor and the substrate were added simultaneously the inhibitory effect was much less than when the inhibitor was added alone and pre-incubated for various lengths of time before the addition of the procaine substrate. Increasing the duration of the pre-incubation tended to increase the inhibitory effect of the same inhibitor concentrations.

Effect of ascorbic acid on oxidation of aromatic drugs in the body. JULIUS AXELROD,* BERNARD B. BRODIE AND SIDNEY UDENFRIEND.* *Section on Chemical Pharmacology, National Heart Institute, National Institutes of Health, Public Health Service, Federal Security Agency, Bethesda, Maryland.*

The finding that a model system containing ascorbic acid can bring about drug oxidations analogous to those occurring *in vivo* has led to investigation of the possible role of ascorbic acid in the metabolism of drugs and other organic compounds. The compounds studied were acetanilide, aniline, antipyrine, and theophylline, which are metabolized in the body largely through hydroxylation. The influence of ascorbic acid on the oxidation of these drugs was determined by comparing their rates of disappearance from the plasma of normal guinea pigs with the rates found in guinea pigs depleted of ascorbic acid. The rate of oxidation of these drugs was markedly diminished in the depleted animals. On repletion with

ascorbic acid the oxidative rate was restored to normal. Isoascorbic acid could not replace ascorbic acid in restoring the rate of oxidation. On the basis of results with tissue slices, it seems that the oxidation of the above drugs occurs mainly in the liver. Liver slices from ascorbic acid depleted guinea pigs show a diminished ability to oxidize acetanilide to *p*-hydroxyacetanilide. These results implicate ascorbic acid as a factor in the hydroxylation of aromatic drugs.

Effect of tumors on distribution of radioactive colchicine. A. BACK AND E. J. WALASZEK (introduced by E. M. K. GEILING). *Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.*

Colchicine, uniformly labeled with Carbon-14, was injected subcutaneously in normal mice and was found to have a pattern of distribution showing the maximum concentration of colchicine in spleen and liver. Heart, brain, and blood were found to be devoid of any colchicine. Intestines and kidney, being the organs of excretion, were found to vary greatly in their content of the drug. This distribution pattern was changed in the tumor-bearing mice. In experiments with different types of tumors, Sarcome-180 (C-57 strain), Spindle cell Sarcoma (hybrid A), transplantable mammary carcinoma (C3H), and Ehrlich ascites tumor, the distribution of colchicine was altered. A complete absence of colchicine in the spleen was a characteristic effect of tumor implantation. The tumors studied all contained a significant quantity of colchicine. Preliminary experiments indicate that injected lyophillized tumors have the same effect on the distribution of colchicine as the tumors growing *in situ*.

A new blood flow meter. IRA MARK BARASH* AND BYRON B. CLARK. *Pharmacology Dept., Tufts College Med. School, Boston, Mass.*

While assembling equipment for the heart lung preparation, the need became apparent for a simple blood flow meter containing few moving parts and relatively independent of viscosity changes. We preferred a meter that would instantaneously record mean flow rather than unit volume increments. The following principle was employed: By equating potential energy of a unit volume of fluid at the top of a vessel to kinetic energy of a unit volume at an orifice at the bottom of the vessel, it becomes evident that fluid height is proportional to the square of the discharge velocity, $mgh = \frac{1}{2} m v^2$; $h = Kv^2$. At a constant inflow rate, the fluid height increases until outflow equals inflow, at which point the fluid height remains constant. To construct our flow meter, a large siliconed test tube with a small hole at the bottom was suspended from a fine steel spring. One end of a lever was attached to the junction of spring and test tube, the other end adjusted to write on a kymograph. The blood flowed into the top of the freely suspended test tube and was collected in a funnel below. Since fluid height is a linear function of weight and a parabolic function of outflow velocity, a perfectly straight calibration line was drawn by plotting the vertical pointer height against the square of the rate of flow. $(Flow)^2 = K$ (height). Factors influencing sensitivity, accuracy and range were studied and calibrations with fluids of varying viscosity were compared. The meter is accurate to approximately 5% and relatively free of viscosity changes.

An electrical method of assaying the effects of drugs on the wound potential of skin. T. C. BARNES. *Dept. of Pharmacology, Hahnemann Med. College and Hosp. of Philadelphia, Philadelphia, Pa.*

The electrical technique devised for determining the healing rate of experimental abrasions (*Am. J. Surg.*, 69: 82, 1945; 82: 720, 1951) has been confirmed by new clinical data. An ulcer on the ankle (7.5 x 5 cm.) had a potential of 60 mv. positive (compared with the intact skin on the foot) which declined in magnitude as the lesion healed. The potential was almost zero 1 month later when the patient was discharged from the hospital. A wound potential of 40 mv. positive was measured on the hand 2 months after burning by an ether explosion (an unsatisfactory skin graft had been made). Skin wheals produced by allergic reaction to rabbit fur were 35 mv. positive. In experimental abrasions on the finger tips 0.5% chlorophyll was found to produce a decline in the day-to-day positive wound potential more rapidly than in controls. The loss in wound potential was 2.43%/hour for chlorophyll and 1.13%/hour in controls. Oxidized cellulose U.S.P. prolonged the decline in wound potential (showing that the electrical method measures epithelization). The wound potential was increased 3 mv. by ether and by chloroform. The substitution of isotonic KCl, $CaCl_2$ or $MgCl_2$ for NaCl had no immediate effect on the wound potential either initially or during the healing process. Forty % NaCl (E.K.G. paste) produced 3–8 mv. negative. Diluting the isotonic KCl or NaCl 3 × with 5% glucose produced a 4-mv. increase in the positive wound potential (reversible).

Coronary dilating action of L-hydrazino phthalazine (BA-5968) (Apresoline) in the canine heart-lung preparation. WALTER BARRETT,* WILLIAM REITZE,* A. J. PLUMMER AND F. F. YONKMAN. *Research Lab., Ciba Pharmaceutical Products, Inc., Summit, N. J.*

The coronary dilating properties of Apresoline (BA-5968) had been demonstrated in the rabbit heart by Gross, Druey and Meier (*Experientia* 6:

19, 1950), but our early attempts to determine such properties in the feline heart yielded equivocal results and hence directed our attention to the cardiac effects in the canine heart-lung preparation (*Federation Proc.* 9: 265, 1950). Following the studies concerning the gross inotropic effects of Apresoline to determine limits of tolerability, we examined the influence of this compound on coronary flow, both on the spontaneously occurring flow and on the reduced flow elicited by the administration of Pitressin (as demonstrated by MEIER, BEIN AND TRIPOD. In press, *Experientia*, 1952). Our results are summarized as follows: *1*) In the dose range of 75 γ–1.0 mg. (total doses in approximately 1000–1200 ml. of blood), Apresoline evoked a slowly developing but progressive coronary dilation which persisted for at least 1 hour; the duration appeared to be a function of the dose employed. *2*) A dose of 200 γ of Apresoline antagonized the coronary vasoconstrictive action of 0.1–0.2 U of Pitressin. *3*) When tested against the sustained vasoconstrictive action of a constant infusion of Pitressin, Apresoline in a total dose of 200 γ or less evoked marked persistent coronary dilation. *4*) Apresoline produced a marked potentiation of the coronary vasodilating action of L-epinephrine and nor-epinephrine. In addltion, potentiation of the positive inotropic action of L-epinephrine occurred, although this effect was much more transient than inactivation of epinephrine-enhanced coronary flow.

Acute and subacute toxicity of ethylene diamine tetraacetic acid (EDTA) salts. ROBERT O. BAUER, FIORE R. RULLO, CHARLES SPOONER AND EARL WOODMAN (introduced by GEORGE L. MAISON). *Dept. of Pharmacology, Boston Univ. School of Medicine, Boston, Mass.*

Considerable interest attaches to abstraction of metals from biologic systems by EDTA insofar as specificity can be obtained. Calcium disodium EDTA (CaEDT) should remove lead and certain other metals from the body with formation of lead disodium EDTA (PbEDT). Toxicity data are needed not only for CaEDT but for PbEDT formed *in vivo*. Acute toxicity determinations with CaEDT is rendered difficult by solubility relations. The i.p. LD_{50}'s in mice, rats and rabbits exceeded 4.5, 7, 6 gm/kg.; i.v. in rabbits was below 4 gm/kg. with no death at 2 gm/kg. Daily (5/week) doses (0.1, 0.2, 0.3, 0.5, 1.0 gm/kg.) i.p. in Wistar rats (6/dose) have produced a graded weight depression during 14 weeks' administration. Daily (5/week) i.p. injections of CaEDT in rabbits (0.5 gm/kg.) and cats (0.25 gm/kg.) resulted in time-50% mortality (LT_{50}) and 95% confidence limits of 78(59–103) and 78(35–170) days. Dogs (20) challenged twice daily with 150, 250 and 500 mg/kg. i.v. produced LT_{50}'s of 40(27–59), 18.5 (12–28) and 13(12–20) days. In rabbits, 1 dose i.v. LD_{50} of PbEDT was 1000 mg/kg. as Pb, whereas the i.p. LD_{50} was 350 mg/kg. The latter value was unexpected in view of the approximate LD_{50} equality of i.p. and i.v. CaEDT. Daily (5/week) i.p. administration, 20 and 40 mg/kg. Pb as PbEDT, was not toxic to rabbits over 105 days. PbEDT in daily doses of 70 and 100 mg/kg. as Pb produced LT_{50}'s of 8.5(5.3–13.7) and 1.3(1.0–1.6) days. Chickens poisoned with metallic Pb deposited in crop sac were protected from weight loss, anemia, ataxia and wing drop when 0.5% CaEDT was included in their drinking water.

Effect of N,N dimethyl 4-piperidylidene 1,1 diphenylmethane methyl sulfate (Prantal) on insulin-induced gastric secretion in subjects with duodenal ulcer. ALEXANDER C. BAYER AND SARAH BRADFORD (introduced by HARVEY B. HAAG). *McGuire Veterans Administration Hosp., Richmond, Va.*

N,N dimethyl 4-piperidylidene 1,1 diphenylmethane methyl sulfate (Prantal) has been shown to inhibit synaptic transmission across certain ganglia in the parasympathetic nervous system in selected animals. This study is concerned with the clinical efficacy of the drug in varying dosages and its effect in the human on the insulin-induced secretion of gastric acid and mucoprotein. Male subjects with active duodenal ulcer were selected for study. Each individual had a preliminary control insulin test and several days later the test was repeated in the same subject 2 hours after Prantal administration. Dosage varied from 100–250 mg. Comparison of the control tests and tests after premedication with Prantal in doses of 100–250 mg. orally reveals no significant alteration in volume, free HCl, total acidity or mucoprotein. Clinical response was variable and unpredictable in all cases. Further study of this compound with larger doses seems indicated in order to determine its efficacy as a cholinergic blocking agent and its value as an adjunct in the therapy of gastroduodenal ulcer. Minimal atropine-like side effects were noted.

Analgesic activity and toxic effects of acetylmethadol isomers in man. HENRY K. BEECHER AND ARTHUR S. KEATS.* *Anesthesia Lab. of Harvard Med. School at Massachusetts General Hosp., Boston, Mass.*

Alpha-acetylmethadol (*dl*-6-dimethylamino-4,4-diphenyl-3-acetoxy-heptane) recently synthesized from methadone has certain curious properties as described by Fraser and Isbell. They found that the levo-isomer (30 mg.) on subcutaneous administration did not produce a morphine-like effect, which they described as 'euphoria', until after some 9 hours, whereas after oral administration

the chain of morphine-like reactions appeared in 1 or 2 hours. The purpose of the present study was to test the analgesic properties of the acetylmethadols. This evaluation was made on postoperative patients in pain by the method of Denton and Beecher, and Keats, Beecher and Mosteller. Three dose levels (subcutaneous) were tested in a total of 81 patients, 5, 20, and 40 mg/70 kg. body weight. The 20-mg. dose satisfactorily relieved the pain of 56% of the patients; this is well below the effectiveness of 10 mg. morphine. Doubling the dose of the acetylmethadol to 40 mg/70kg. did not increase the analgesic power of the agent. A comatose state was produced in 4 out of 45 subjects. One of the 4 had a transient period of Cheyne-Stokes respiration and 1 had a typical morphine-like depression of respiration. There was no depression of respiration in the other 2 cases. The total dose of levo-acetylmethadol in the 4 cases was as follows: *1*) 16 mg. twice in 30 hours, with 8 mg. morphine twice in the same period; *2*) 32 mg. twice in 25 hours with 8 mg. morphine 3 times in the same period; *3*) 40 mg. twice in three-fourths hours with 10 mg. morphine once in this period, and *4*) 26 mg. twice in 30 hours with 6 mg. morphine once in this period. The delayed morphine-like actions of the levo-isomer offer promise that it may be possible to separate the important side effects of powerful narcotics from their analgesic power.

Vasodepression produced by an anticholinesterase agent. CARL W. BENDER,* B. P. MCNAMARA AND J. H. WILLS. *Pharmacology Branch, Chemical Corps Med. Labs., Army Chemical Center, Md.*

The intravenous administration of anti-cholinesterase drugs produces a precipitous decrease in blood pressure. It is considered that this is mediated largely through peripheral mechanisms. However, the removal of central tonal impulses following failure of neural transmission is also a possibility. The blood pressure changes produced by an anticholinesterase compound in anesthetized, artificially respired cats was studied in the following groups of animals: *1*) double vagotomy, *2*) high spinal section, *3*) high spinal section plus vagotomy, *4*) controls having low blood pressure produced by exsanguination, *5*) controls with normal blood pressures. The vasodepression produced by the agent was less in *group 3* than in *groups 1*, *2*, *4* and *5*, suggesting that there is an important central factor in the appearance of the blood pressure fall after injection of some anticholinesterases.

Comparative pharmacology of D-, DL- and L-Dromoran and related ether derivatives. W. M. BENSON,* P. L. STEFKO* AND L. O. RANDALL. *Dept. of Pharmacology, Hoffmann-LaRoche, Inc., Nutley, N. J.*

Comparative studies of Dromoran and its optically active antipodes have demonstrated that the analgesic activity of Dromoran, as determined by the prolongation of reaction time in rats, resides in the L-isomer. The D-isomer has negligible, if any, analgesic activity. The respiratory depressant activity of L-Dromoran, as measured in unanesthetized rabbits, as well as the toxicity, as evaluated in mice and rats, is greater than that of D-Dromoran. The action of racemic Dromoran is intermediate in all these respects. All 3 agents exhibit moderate antitussive activity in the unanesthetized dog, in which coughs are induced by the electrical stimulation of the trachea. The D-, L- and DL-methyl ether derivatives of Dromoran exhibit parallel analgesic characteristics, although they are less potent and more toxic than the corresponding parent compounds. The L-methyl ether of Dromoran is a respiratory depressant, although less potent than levo-rotatory Dromoran, and the D-methyl ether of Dromoran stimulates respiration in low dosage. The respiratory activity of the DL-methyl ether of Dromoran is intermediate between that of the D- and the L-isomers. Antitussive activity also resides in the methyl ethers of Dromoran. Higher ether derivatives of this series are currently being studied.

Medicinal uses of alginic acid and of its salts. F. M. BERGER AND B. J. LUDWIG.* *Wallace Labs., Inc., New Brunswick, N. J.*

Alginic acid differs from other materials extracted from seaweeds, such as agar or Irish moss, in possessing a reactive carboxylic group. Thus it can be used for adsorption of sodium in the intestinal tract in place of the cation exchange resins. *In vitro* alginic acid at pH 7.6 adsorbs 4.2 mEq. Na^+/gm. As is the case with resins, the ratio of Na:K adsorbed approximates that present in solution. Alginic acid differs from the resins in forming soluble and hydrophilic sodium and potassium salts. The calcium alginate is insoluble. Sodium alginate possesses greater water absorbing and water retaining properties than methylcellulose or natural hydrophilic gums. Swelling does not occur in acid environment. These properties make sodium alginate very suitable for use as a bulk laxative. Sodium alginate also possesses acid neutralizing and buffering action. When to 2.5 gm. of sodium alginate 25-ml. portions of N/10 HCl were added at 30-minute intervals according to Hammerlund (*J. Am. Pharm. Assoc.* 38: 586, 1949), an immediate and sustained neutralizing action was observed. Alginic acid maintained the reaction in the region of pH 2.5–pH 3.0. The ability to neutralize acid without alkalization together with the known hemostatic properties of alginates may make them valuable in the treatment of ulcers.

A universally useful absorption base for ointments. REINHARD R. BEUTNER. *New England College of Pharmacy, Boston, Mass.*

This new type of absorption base can be used in order to make ointments by incorporating watery, oily, or fatty solutions, or substances soluble in water, oil or fat. Dissolution or emulsification occurs whereas the absorption bases, used so far, fail to mix with all of these solutions; e.g. Aquaphor or other lanolin-containing ointment bases fail to mix with alcohol. Jellified alcoholic solutions, like collodion, do not mix with most fats or water. The action of topical medicaments, when dissolved in the new ointment base, is intensified. Staining drugs like coal tar or chrysarobin, when added to the new base, are held in place after application to the skin, so that a non-staining ointment is obtained. From a pharmacological standpoint this new ointment is of interest since it also alters the mode of action of added medicaments in some cases; e.g. camphor, when added at 5%, has a marked analgesic effect while it is much more irritant and less analgesic in some other ointments, at that concentration. This point can be demonstrated by experiments on the rabbit's eye. The new ointment base is made by first preparing an emulsion in water, of an alcohol-soluble resin. This emulsion is formed by mixing the alcoholic solution of the resin with a solution of an emulsifier like acacia. The watery alcoholic emulsion so obtained is then jellified by the addition of a hydrophilic colloid like starch. A fat-emulsifying substance like lanolin or lecithin is finally added. All materials from which an ointment base of this type can be made are readily available.

Electrical action of drugs as the cause of their depressant or stimulant effect. REINHARD H. BEUTNER. *New England College of Pharmacy, Boston, Mass.*

The electrical theory postulates that drugs work by direct electrical action on phase boundaries existing in tissue between lipids and watery fluids. Previous investigations have demonstrated the marked electrical effects of potent drugs on phase boundaries of water and lipids (R. Beutner). Both stimulants and depressants shift the potential differences *in vitro* to the negative side. A distinction between stimulants and depressants in regard to their electrical effect *in vitro* seems to be that the depressants have a more persistent action (personal communication by T. C. Barnes). If this also occurs *in vivo* the positive charge of the nerve fiber is permanently abolished. This charge is indispensable for the propagation of the impulse as emphasized by Lillie's local circuit theory. It should therefore be possible to lift the block of a depressant by any substance which again restores the positive charge, in other words, shifts the potential difference back to the positive side. That this actually occurs was demonstrated in experiments on the isolated intestine of rabbits suspended in Tyrode solution. Constriction occurred when pilocarpine 1:1 million was added. Addition of atropine 1:2 million blocked this action. Now, the gut was treated with sodium benzoate 1:10,000 which has no effect on the gut by itself, but according to *in vitro* experiments is known as one of the substances which shift potential differences to the positive side. Sodium benzoate has apparently the same effect *in vivo* since it was observed to lift the atropine block, thus restoring intestinal responsiveness to pilocarpine. The theory is thus confirmed.

Synthesis of glucuronic acid. THOMAS G. BIDDER* (introduced by LAWRENCE PETERS). *Dept. of Pharmacology, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

The synthesis of menthol glucuronic acid by liver slices of fasted guinea pigs was studied using glucose-1-C^{14} and lactate-3-C^{14} as substrates in an attempt to clarify the role of these compounds in the synthesis of conjugated glucuronides. The menthol glucuronic acid thus formed was isolated and degraded with buffered periodate, which yielded carbon 1, carbons 2, 3 and 4, and carbons 5 and 6 as separate fractions, and by decarboxylation with 12% HCl, which gave carbon 6 as a separate fraction. When glucose-1-C^{14} is used as the labeled substrate, the radioactivity appears largely in the 1 position of the glucuronic acid with no major redistribution to the other positions in the molecule. With lactate-3-C^{14} as the labeled substrate, the specific radioactivity is highest in carbons 1 and 6 of the glucuronic acid molecule, but the specific activity of carbon 6 is 2.5 times that of carbon 1, indicating asymmetric incorporation of the C^{14} in the two halves of the glucuronic acid molecule. These data, therefore, do not exclude the possibility that, in the biosynthesis of conjugated glucuronic acid, a triose, produced from lactate, condenses with another triose formed from glucose and that, in this manner, carbons 1, 2 and 3 of the glucuronic acid originate predominantly from carbons 1, 2 and 3 of glucose, while carbons 4, 5 and 6 originate from a three-carbon compound. (This study was supported, in part, by a grant from the Nutrition Foundation, Inc.)

Effects of histamine on renal functions. WILLIAM BLACKMORE AND T. R. SHERROD (introduced by C. C. PFEIFFER). *Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago.*

The action of histamine on glomerular filtration rate and renal plasma flow was determined in

trained, unanesthetized female dogs by the technics of inulin and para-aminohippuric acid clearances. Histamine in doses of 2.5 μg/kg. of body weight/minute was administered by constant intravenous infusion. Each experiment was divided into 4–8 periods for the control studies and into 8 periods for the histamine studies. Thirteen control experiments and 21 histamine experiments have been performed. Continuous blood pressure readings were done in some of the experiments. All animals which received histamine demonstrated the usual reactions of salivation, flushing, defecation and occasionally vomiting. Statistically significant increases in renal blood flow were observed in each dog which received histamine. This averaged about 30% above the control values during the first hour. No significant changes were noted in glomerular filtration rate nor in the filtration fraction. The urine flow was markedly reduced during histamine infusion. The depressor responses to histamine varied between 70 and 80 mm. Hg (control b.p. of 110–120). During the first hour following the cessation of histamine infusion, the renal blood flow was still elevated to the extent of 15–20%. Also, during this period the blood pressure returned to normal and the rate of urine flow exceeded control values.

A photochemical color reaction of biotin with ninhydrin in sulfuric acid. THOMAS R. BLOHM AND CAROL M. VOLKMANN. (introduced by C. A. HANDLEY). *Dept. of Biochemistry, Univ. of Texas, Dental Branch, Houston.*

A photochemical reaction between ninhydrin and biotin in concentrated sulfuric acid has been discovered. The reaction produces color by means of which somewhat less than 0.1 of a mg/ml. of biotin can easily be detected. Heat also causes a reaction which appears to be similar to that produced by light. Absorption curves for products of both reactions bear out this conclusion. The presence of the sulfide structure in biotin appears to be necessary for color production, but evidently other parts of its structure are also important, since other sulfides such as methionine and tetrahydrothiophene are much weaker color producers. Ninhydrin and sulfuric acid have just been shown to be a color reagent for aromatic hydrocarbons (in press), with which compounds this reagent produces halochromic salts, but only in the cases of the sulfur compounds mentioned has photolabilization been noticed. Limiting conditions for the reaction and its specificity have been investigated and will be reported.

Diparcol in nicotine poisoning. DONALD F. BOGDANSKI AND JOHN A. DATTOLI (introduced by THEODORE KOPPANYI). *Dept. of Pharmacology, Georgetown Univ. Med. School, Washington, D. C.*

Albino mice were injected with 1% solution of nicotine salicylate subcutaneously in the abdominal region. The LD_{50} was 55 mg/kg. and the LD_{99} about 70 mg/kg. It is necessary to state the percentage strength of the solution injected by this method, otherwise statements concerning LD_{50} or other standard doses lose their significance. Larger doses of nicotine were necessary to produce the desired end points when more dilute solutions were employed (due to absorption of less drug per unit time, although the rate of absorption of the injected fluid may be the same). General symptoms of nicotine toxicity include motor excitement, hyperpnea, clonic convulsions, dyspnea and finally tetanic spasms. Death occurs usually within 5–10 minutes. If death does not occur within this time the animals generally survive. Diparcol, 30 mg/kg. (intramuscular injection) protected against 70 mg/kg. of nicotine. All symptoms were diminished in the protected animals; clonic convulsions occurred but tonic spasms were completely abolished. Diparcol in doses up to 60 mg/kg. did not protect against 120 mg/kg. of nicotine salicylate. In these cases tonic convulsions preceded death. Atropine in doses of 30 mg/kg. protected 50% of the mice receiving 70 mg/kg. of nicotine. Anesthetic doses of Nembutal had little or no effect on the LD_{50} of nicotine, but the combined administration of 25 mg/kg. Nembutal and 30 mg/kg. Diparcol was occasionally effective in saving the lives of mice receiving 120 mg/kg. nicotine.

Elevation of the cardiac depressant dose of a barbiturate with cardiac stimulant drugs. KENNETH J. BONIFACE AND JOHN M. BROWN (introduced by ROBERT P. WALTON). *Depts. of Pharmacology and Surgery (Anesthesiology), Med. College of South Carolina, Charleston.*

Under artificial respiration and a constant rate of barbiturate infusion, cardiac arrest doses were determined in 28 dogs. Following anesthesia with 30 mg/kg. pentobarbital sodium, this drug was mechanically infused at the rate of 5 mg/kg/min. until cardiac arrest. Artificial respiration was instituted at respiratory paralysis. The point of cardiac arrest or near cardiac arrest was determined by pulse pressure recordings from the carotid artery with a Statham pressure transducer. Respiratory paralysis occurred at an average total dose of 50 mg/kg. (S.D. ± 10) and cardiac arrest at 143 mg/kg. (S.D. ± 46). In 6 experiments, a sympathomimetic amine (2-methylamino-6-hydroxy-6-methyl heptane) (Aranthol) was administered when cardiac arrest was imminent and subsequent injections given when needed. The average cardiac arrest dose in such cases was increased to 486 mg/kg. (S.D. ± 105) using an average dose of 85 mg/kg. (S.D. ± 18) Aranthol. In 5 other experiments ouabain (0.5 cat units/kg.) was administered previous to the

start of the pentobarbital infusion; the average cardiac arrest dose in such cases was 224 mg/kg. (S.D. ± 53). When pentobarbital infusions were discontinued after 2–3 cardiac depressant doses had been antagonized with Aranthol, recovery occurred to the level of return of eye reflexes and, in one case, spontaneous movement; death occurred in these cases, however, due to pneumonia and other secondary complications.

Nodose ganglion as receptor site for emetic and vasodepressor actions of veratrum. HERBERT L. BORISON AND VIRGIL F. FAIRBANKS (introduced by LOUIS S. GOODMAN). *Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City.*

The nodose ganglion has been shown to be the site of emetic action of veratrum alkaloids by Fairbanks and Borison (*J. Pharmacol. & Exper. Therap.*, 103: 343, 1951). Further evidence to support this claim was obtained as follows. Three cats (tracheotomized) with chronic excision of both nodose ganglia failed to vomit in response to large doses of Veriloid, but two of these cats vomited following lanatoside C and lobeline, respectively. The emetic response to Veriloid was unaltered in 2 cats with chronic bilateral decapsulation of the nodose ganglion. In one of these cats Veriloid emesis was elicited following decerebration and vagal section below the nodose ganglia but not after vagal section above these ganglia, although lanatoside C still induced vomiting. Recent reports of central hypotensive action of veratrum, demonstrated in cross perfusion experiments, have not excluded the nodose ganglion as a possible receptor site. In our experiments on cats, hypotensive responses were induced by Veriloid after mid-cervical bilateral vagotomy but such responses could not be elicited after vagotomy above the nodose ganglia. Indeed, the effect of the drug after exclusion of the nodose ganglion was usually a rise in blood pressure. (Research supported by grant from Committee on Research, American Medical Association.)

Effects of emulsifying agents upon intestinal content of the white rat. A. R. BOURKE,* J. P. FRAWLEY* AND O. G. FITZHUGH. *Div. of Pharmacology, Food and Drug Admin., Federal Security Agency, Washington, D. C.*

A comparative study of some pharmacological properties of sorbitan monolaurate, polyoxyethylene sorbitan monolaurate, glyceryl monolaurin, glyceryl trilaurin, sorbitan monostearate, polyoxyethylene stearate, polyoxyethylene sorbitan monostearate, glyceryl monostearin, glyceryl tristearin, sorbitan monooleate, polyoxyethylene sorbitan monooleate, glyceryl monoolin, glyceryl triolein, glyceryl monopalmatin, glyceryl tripalmatin, super glycerinated fat containing 90% monoglycerides and a 30–40–30% mixture of mono, di and tri glycerides respectively is reported. Twenty-five per cent lipid diets were prepared in Purina Laboratory Chow base. Two diets, Purina Chow and Purina Chow with 25% Crisco, served as controls. Five male and 5 female weanling rats were placed on each of the above diets, housed individually and rat weights and food intake were recorded weekly for 8 weeks. Feces were examined for microscopic character, occult blood, intestinal flora, total quantity and total lipids. After 8 weeks animals were bled for chemistry and sacrificed for gross pathological examination. The effects of 17 lipid diets upon growth response and character of intestinal contents and bacterial flora will be compared with effects of normal diet and high fat content diet.

Radiation effects in isolated organs. I. Effects of x-radiation on bile flow, performance and survival in isolated rat liver and comparison with several vasoactive agent. RALPH W. BRAUER, G. F. LEONG* AND R. J. HOLLOWAY.* *Biological and Med. Sciences Div., U. S. Naval Radiological Defense Lab., San Francisco, Calif.*

A number of noxious agents have been found effective in impairing or arresting bile production by the isolated liver. x-radiation (500–800 r) is peculiar among these in that its effects are delayed. Bile flow rates begin to decrease 30–60 minutes after exposure, and dwindling progressively, cease entirely within 180 minutes after radiation. To determine whether this phenomenon can be ascribed to release of pharmacologically active substances by irradiated cells, the effects of several known agents which might be involved, including acetyl choline, epinephrine, histamine, and ATP, have been analyzed and compared with those due to radiation. Blood and bile flow, organ weight, bromsulfalein uptake and excretion, recorded continuously, and intermittent determination of bile secretion pressures have been employed in this work. Studies will be reported involving the use of multiple coupled circulations to investigate the effect of fresh blood upon irradiated organs, or of blood from irradiated livers upon fresh organs. The data suggest that the effect of radiation on the isolated liver represents direct cellular injury rather than a humoral phenomenon.

Comparative study of the USP XIV colorimetric assay and the USP XIII pigeon method in assay of digitoxin. HERBERT A. BRAUN AND L. M. LUSKY* *Div. of Pharmacology, Food and Drug Admin., Federal Security Agency, Washington, D. C.*

Assays by both the USP XIV colorimetric method and the USP XIII intravenous pigeon method for determining the potency of digitoxin have been completed on over 30 samples consisting

of pure digitoxin and of digitoxin tablets. The tablets were extracted by the method described in the USP XIV and the same extract, after proper dilution, was used for both determinations. At least 4 determinations were carried out for each sample by the USP XIV method and duplicate assays were made by the USP XIII method were wide differences existed between the 2 methods. In 20% of the samples examined the potency by the colorimetric method was 125% or more of that found by the biological assay. In one case the potency was 218% of the biological. It is clear that the 2 methods do not always give concordant results.

Mechanism of production of metabolic acidosis and hyperglycemia during ether anesthesia in the dog. WILLIAM R. BREWSTER,* JOHN P. BUNKER* AND HENRY K. BEECHER. *Anesthesia Lab., Harvard Med. School at the Massachusetts General Hosp., Boston.*

Ether anesthesia in the dog has been shown to result in a moderately severe metabolic acidosis with rise of total fixed acids, lactic acid and pyruvic acid in the blood. This acidosis is accompanied by a hyperglycemia and by a consistent fall in serum potassium levels. Although the normal adult man does not show a significant rise in fixed acids during ether anesthesia, the infant and child develop an acidosis that is similar in all respects to that occurring in the dog, although somewhat less severe. Ether anesthesia was administered to 17 dogs in which a total sympathetic block had been established and maintained by the intermittent injection of 0.4% procaine into the epidural space. In the dogs with a total sympathetic block, ether anesthesia at arterial levels of 95 mg.% failed to produce a metabolic acidosis and no significant changes in blood lactic acid, pyruvic acid, total fixed acids, blood sugar or serum potassium were observed. The metabolic effects of ether anesthesia mentioned above were duplicated by infusing, into the dog with a total sympathetic block, quantities of epinephrine that may be physiologically liberated from the adrenal medulla and sympathetic nerve endings during ether anesthesia. It is concluded that the metabolic acidosis and hyperglycemia of ether anesthesia in the dog are directly related to the quantitative output of epinephrine occurring during ether anesthesia with resultant mobilization by epinephrine of liver glycogen as glucose and muscle glycogen as lactic acid. From the above work in the dog, we may infer that the presence of a metabolic acidosis in the human child during ether anesthesia, as contrasted to the absence of a metabolic acidosis in the normal adult, is due either to a greater relative output of epinephrine in the child during ether anesthesia as contrasted with the adult or to a greater sensitivity of the child to epinephrine.

Vasodepressor effects of epinephrine and arterenol in cerebral perfusion. J. BAYARD BRITTON* AND RAYMOND P. AHLQUIST. *Med. College of Georgia, Augusta.*

Taylor and Page (*Circulation*, 4: 563, 1951) have reported that epinephrine or arterenol when injected into the arterial supply to the perfused dog head produce a marked depressor response in the body which is connected to the head by only the spinal cord, the cerebral circulation being maintained from a donor animal through a carotid artery and jugular vein. We have confirmed, and extended, this work using their technique with some modifications, one of which was the measurement of the arterial perfusion flow using an optical rotameter. Blood flow through the internal carotid was not significantly changed until the drug had passed through the perfused head and returned to the donor; flow then increased due to a pressor response. The magnitude of both the depressor response in the recipient body and the pressor response in the donor was determined by the circulation rate through the head. This rate was determined primarily by the adequacy of the venous return, which in turn was determined by the size of the cannulae and connecting tubes used. When circulation through the head was slow the depressor response was large and the donor's pressor response small. With adequate venous return, the depressor response was small and the pressor response large, equalling that produced by the intravenous injection of the same dose of epinephrine or arterenol. Evidence has not been obtained which would indicate that this central depressor mechanism plays a significant role in the intact animal. (Supported by a grant from the American Heart Association.)

Oxidation of aromatic drugs by ascorbic acid, oxygen and ferrous iron. BERNARD B. BRODIE, PARKHURST A. SHORE,* JULIUS AXELROD* AND SIDNEY UDENFRIEND.* *Section on Chemical Pharmacology, Natl. Heart Inst., National Insts. of Health, Bethesda, Md.*

A model system containing ascorbic acid, oxygen, ferrous iron, and ethylenediamine tetraacetate, a metal complexing agent, has been shown to oxidize tyramine to hydroxytryamine. Investigations of this system were extended to a number of other aromatic compounds. In each case, the oxidation products were identical with those which have been isolated from urine after the administration of the compounds to man. Thus: acetanilide → p-hydroxyacetanilide; aniline → *p*-aminophenol; antipyrine → 4-hydroxyantipyrine; salicylic acid → gentisic acid; quinoline → 3-hydroxyquinoline; theophylline → 1,3-dimethyluric acid. The hydroxyl group invariably appeared in a position in the ring possessing a net negative charge, suggesting the replacement of ring hydrogen by OH^+. The

yield of the hydroxylated derivative was large enough, in some cases, to suggest the use of the ascorbic acid oxidative system as a synthetic tool for the direct introduction of a hydroxyl group. Interestingly enough acetophenetidin (p-ethoxyacetanilide) yields p-hydroxyacetanilide suggesting that this ether is de-ethylated through an oxidative mechanism. The implications of the above findings in the metabolism of drugs will be discussed.

Mechanism of mercurial diuresis in hydropenic dogs. WILLIAM A. BRODSKY AND HARRY N. GRAUBARTH (introduced by P. K. KNOEFEL). *Dept. of Pediatrics, Inst. for Med. Research, Univ. of Louisville, Louisville, Ky., and Dept. of Pediatrics, Children's Hospital Research Fndn., Univ. of Cincinnati, Cincinnati, Ohio*

Mercuhydrin, in doses of 5–9 mg/kg. of body weight, was administered intravenously to barbitalized dogs. They had been deprived of water for 20 hours and food for 10 hours preceding experiments, and were excreting urine at flows of 0.12–0.33 cc/min/m². with osmotic activities of 1376–1658 mosm/l. Urinary flow increased rapidly 45 minutes after injection attaining values as high as 8.0 cc/min., while osmotic activity, falling to 400–500 mosm/l., remained hypertonic to plasma. Flows remained at maximal levels for 30 minutes, and gradually decreased to hydropenic levels in the next 90–100 minutes. In plasma, osmotic activity and composition of solutes were unchanged during preliminary and diuretic periods. During diuresis, the relationship between urinary flow and effective osmotic load was characteristic of the constant pattern found in hydropenic diuresis forced by a wide variety of loading solutes. Na and Cl, reaching concentrations of 200–300 mEq/l. each, accounted for 80–85% of urinary solutes. The rate of Na loss was as high as 1.2 mEq/min/m². Clearances of inulin and PAH, when measured, were essentially unchanged. It appears that mercurial action, at a proximal tubular site, causes a large solute load, mainly Na and Cl, to be presented to the distal tubule. The distal tubule responds osmometrically, elaborating a final urine, the volume of which, like that in any osmotic diuresis, is dependent on the number of osmotically active particles claiming excretion.

Further correlations of behavior with voluntary choice of alcohol in albino rats. ROBERT G. BROWN,* WILLIAM T. MOORE*, BERNICE M. MOORE* AND G. A. EMERSON. *Univ. of Texas Med. School, Galveston.*

Genetotrophic aspects of alcohol preference in rats have been noted by R. J. Williams *et al.*, while correlations with other forms of behavior have not been as extensively studied in lower animals as in man. Objective measurements of learning ability and memory in maze running performance, aggressive behavior, motor activity, fear of cats, thresholds to electroshock, metrazol and insulin, and susceptibility to audiogenic seizure have been made, and the results interpreted in terms of behavior patterns related to high voluntary intake of 5 or 10% V/V ethanol solutions.

Dose response curves for pressor effects of pure L-norepinephrine, L-epinephrine, and a commercial epinephrine. ROBERT V. BROWN. *Div. of Pharmacology, Univ. of Tennessee, Memphis.*

In a previous report (*Federation Proc.*, 10: 283, 1951) a method of describing the dose response curves, which are rectangular hyperbolas, in terms of 3 parameters was developed. Additional analysis indicates that after translation of both axes, the curves can be defined in terms of a single parameter, when the units of dose and response are given. Each curve then has the equation $x'y' = -k$, where x' represents the dosage corrected for the drug equivalent of the control blood pressure, y' is the failure to attain the asymptote and $k = ab + c$. The asymptote parallel to the X (dosage) axis is $y = a$; the asymptote parallel to the y (response) axis is $X = -b$. The constant of integration, c, corrects for error in the control blood pressure level. Geometrically, k is the area of a rectangle having portions of the x' and y' axes as two sides, one vortex at the origin and the diagonally opposite vortex at any point on the dose response curve. By definition, k is the absolute potency of the drug; $\sqrt{k}$ is the dose or response potency. The sensitivity of the cardiovascular system to the drug is defined as the first derivative of the dose response curve. A method of stating potency ratios requiring no assumptions of equal dosage, equal responses or equal slopes is thus possible. Certain implications of the theoretical response curves will be presented for discussion.

Comparison of antiepileptic drugs in mice and rats. WILLIAM C. BROWN,* LOUIS S. GOODMAN AND EWART A. SWINYARD. *Dept. of Pharmacology, Colleges of Medicine and Pharmacy, Univ. of Utah, Salt Lake City.*

Antiepileptic drugs were compared in mice and rats for their ability to abolish the hindlimb tonic extensor component of maximal electroshock seizures (M.E.S.), to elevate minimal electroshock seizure threshold (M.E.T.), to raise experimentally lowered threshold in hyponatremic animals (H.E.T.), and to prevent metrazol convulsions. Thiantoin, diphenylhydantoin, phenobarbital, trimethadione and paramethadione were given subcutaneously; mesantoin, mebaral and phenurone, orally. Minimal toxic doses (TD_{50}) and effective doses (ED_{50}) were determined at the time of peak drug effect and protective indices (P.I. = TD_{50}/ED_{50}) were calculated. The data indicate a differ-

ence in time of peak drug effect in mice and rats, particularly for diphenylhydantoin and phenurone. TD_{50}s are significantly different in mice and rats, but the compounds are not uniformly more toxic in one species. Relative ranking of TD_{50}s was essentially the same in the 2 species. Most compounds were less potent in mice by the M.E.S. test but the order of relative potency was remarkably similar in the 2 species. Potencies as determined by the M.E.T. and H.E.T. tests were not uniformly greater in one species. Except for trimethadione, the compounds had greater antimetrazol activity in mice. It is concluded that when these compounds are assayed at time of peak activity, their profiles of anticonvulsant activity are similar in the 2 species. Until the clinically predictive value of these laboratory tests is firmly established, it seems prudent to use more than one species for the definitive evaluation of candidate antiepileptics. (Research supported by grant from National Institutes of Health, P.H.S.)

Effect of quantity of carrier gallium on distribution of metal in rats. H. D. BRUNER, JESSE D. PERKINSON, JR.,* AND R. L. HAYES.* *Oak Ridge Inst. of Nuclear Studies, Oak Ridge, Tenn.*

Previous work established that Ga^{72} labeled $Ga^{69, 71}$ in doses of 0.5 to 26.0 mg/kg. was differentially distributed among the tissues of adult male white rats in a pattern quite different from carrier-free Ga^{67}. Following intravenous administration of the citrate complex of $Ga^{(69, 71)+72}$ at pH 7.4, the metal was 4 times more concentrated in bone than in liver, spleen, or kidney at 24 hours and 12–14 times at 96 hours. Following carrier-free Ga^{67} (dose of metal less than 2×10^{-7} gm/kg.), the spleen throughout segregated Ga^{67} more than bone while the liver, initially higher, became about equal with bone at 120 hours. Also the excretion patterns distinctly differed. The quantity of carrier $Ga^{69, 71}$ was identified as the variable responsible for the changed distribution patterns. Groups of 4 rats received increasing doses of carrier $Ga^{69, 71}$ (0.0, 0.0025, 0.025, 0.25, 2.5, and 25.0 mg/kg.) mixed with a constant trace dose of carrier-free Ga^{67} which was brought to constant volume and mol ratio with citrate and saline at pH 7.4. Total excretion of Ga over the next 5 days was linearly related to the dose when plotted on log-log paper; the urine/feces ratio shifted from 1.0 to 10 with increase of dose. The differential absorption ratios of the spleen and liver sharply decreased above the 0.25 mg/kg. dose while the bone sharply increased giving an inversion effect. On log-log paper the absolute amounts of metal retained by the femur increased linearly with dose, while the spleen and liver curved horizontally above 0.25 mg/kg.

Metabolic studies in infants and children during anesthesia. JOHN P. BUNKER,* WILLIAM R. BREWSTER,* ROBERT M. SMITH* AND HENRY K. BEECHER. *Anesthesia Lab., Harvard Med. School at the Massachusetts General Hosp., Boston.*

Acid-base and other metabolic equilibria were studied in infants and children during ether and during cyclopropane anesthesia. Serum pH, carbon dioxide content, and hemoglobin were determined in all cases, and in many cases serum lactic and pyruvate acids, sodium, potassium, and chloride and blood sugar were also determined. During ether anesthesia infants under 2 years, unlike adults, regularly developed a moderate metabolic acidosis (drop in corrected bicarbonate of 3-7 mM/l). Between 3 years and puberty, again unlike adults, metabolic acidosis was observed frequently (about 60% of cases). This metabolic acidosis was regularly accompanied by a rise in lactic acid sufficient to account for more than half the calculated change. There was no respiratory acidosis during ether anesthesia. During cyclopropane anesthesia in infants and children, like adults, there was often a slight to moderate respiratory acidosis, but no metabolic acidosis or rise in lactic acid. Serum potassium regularly dropped during ether anesthesia and usually rose during cyclopropane. Changes in chloride and sodium ions were inconsistent and slight in magnitude. The changes in bicarbonate, lactic acid, lactate-pyruvate ratio, blood sugar, and serum potassium during ether anesthesia are qualitatively similar to changes observed following the administration of epinephrine in physiological quantities. They do not resemble the changes observed during anoxia, as has been previously suggested.

Conversion of ethanol to acetaldehyde in vitro. T. N. BURBRIDGE*, V. C. SUTHERLAND* AND CHARLES H. HINE. *Div. of Pharmacology and Experimental Therapeutics, Univ. of California, San Francisco*

Evidence is presented to establish the assumption that acetaldehyde is an intermediate product in the metabolism of ethanol *in vitro*. $CH_3C^*H_2OH$ was incubated with the rat liver slices for 2 hours at 37°C. in Krebs-Ringer solution containing 0.05% semicarbazide hydrochloride. Acetaldehyde formed from the ethanol was identified as the semicarbazone spectrophotometrically and by its melting point. Origin of the labeled acetaldehyde from the labeled ethanol was established following combustion by use of the helium tube scaler.

Effect of acetyl-β-methylcholine, carbamylcholine and atropine on survival of x-irradiated mice. W. T. BURNETT, JR., A. W. BURKE, JR., AND A. C. UPTON (introduced by H. D. BRUNER).

Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.

The ability of certain drugs which act on the autonomic effector cells to modify the effects of x-radiation in mice of the C_{57} black strain has been examined. The 28-day LD_{50} of x-rays for 8- to 10-week-old male mice of this strain is approximately 600 r. The LD_{50} after treatment with acetyl-β methylcholine bromide (mecholyl bromide, 17–135 mg/kg. body weight), acetyl-β-methylcholine chloride (mecholyl chloride, 36 mg/kg.), or carbamylcholine chloride (Doryl, 1 mg/kg.), was approximately 900 r. The protective effect was observed when the drugs were given by intraperitoneal injection 3–5 minutes before total body x-irradiation. A similar pretreatment with betaine (500 mg/kg.), sodium bromide (14 mg/kg.), sodium chloride (0.2 ml. of physiological saline), or atropine sulfate (2 mg/kg., intramuscularly) failed to alter the survival pattern. However atropine, given before irradiation, counteracted the protective action of mecholyl bromide. Gross anatomical findings in mice sacrificed at intervals following x-irradiation indicate the development of acute radiation injury qualitatively similar in both mecholyl-treated and nontreated animals. The damage in the treated group however appeared less severe and recovery occurred much earlier. However, mice that survived 56 days or longer after exposure showed testicular atrophy, depigmentation of fur, and retarded growth indicating that regeneration was not complete. The mechanism of the protective action of the parasympathomimetic drugs remains to be established. Available data suggest that it involves the production of hypoxia, although the possibility of a specific pharmacologic or homeostatic effect must be considered.

Metabolic fate of Tromexan in man. J. J. Burns,* Murray Weiner,* Shirley Wexler* and Bernard B. Brodie. *New York Univ. Research Service, Goldwater Memorial Hosp., New York City, Nat. Heart Inst., National Insts. of Health, Bethesda, Md.*

Tromexan (a carboxyethyl ester of Dicumarol) has been recently introduced as an anticoagulant with a short duration of action. The drug is rapidly metabolized, and negligible amounts are excreted in urine after intravenous administration. The compound is not de-esterified in man; instead an important metabolic pathway involves the introduction of a phenolic group in one of the benzene rings. The position of the phenolic group was determined to be meta to the lactone bridge. The metabolite administered to man was found to exert no effect on the prothrombin time. Only after Tromexan has disappeared from the body does an appreciable amount of the metabolite appear in urine. Studies on patients with biliary fistula suggest that this delayed excretion results from the secretion of the metabolite into bile, followed by the subsequent appearance in urine after absorption from the gastrointestinal tract. Other species metabolize Tromexan differently than does man. The rabbit excretes a considerable fraction of the drug unchanged; the compound does not undergo oxidation in the aromatic ring, but a major route of metabolism involves deesterification to yield the corresponding acid of Tromexan. In the dog the drug does not have a short duration of action; it behaves differently than in man or rabbit in that its metabolic transformation is almost as slow as Dicumarol.

Action of adrenergic blocking agents on nicotine-induced tremors. Raymond L. Cahen and Joan M. Thomas.* *Maltbie Lab., Morristown, N. J.*

In unanesthetized rabbits, nicotine (1 mg/kg. tartrate intravenously) induces tremors which are blocked by anti-Parkinson drugs but not by ganglionic blocking agents and atropine (Bovet and Longo, *J. Pharmacol. & Exper. Therap.* 102: 22, 1951). Such tremors, however, are also blocked by neuromuscular blocking agents and central depressants (Cahen and Lynes, *J. Pharmacol. & Exper. Therap.* 103: 44, 1951). It was found that such tremors are blocked by adrenergic blocking agents 883F, 933F and Dibenamine. A linear relationship exists between the probit of rabbits protected and the logarithm of the doses of the antagonist. The average dose protecting 50% of the animals is 883F, 1.5 mg/kg., 933F, 2.5 mg/kg. and Dibenamine .4 mg/kg. Similar tremors were induced by intravenous injection of a ganglionic stimulant agent 1-Dimethyl-4-Phenylpiperazinium Iodide (500 gamma/kg.) and acetylcholine (15 gamma/kg. after atropine 10 mg/kg. and eserine 1 mg/kg.); such tremors are also blocked by the adrenergic blocking agents.

Parasympatholytic and vagolytic actions of synthetic local apesthetics. Benjamin Calesnick and N. H. Smith.* *Dept. of Pharmacology, Hahnemann Med. College, Philadelphia, Pa.*

The parasympatholytic as well as the vagolytic properties of synthetic local anesthetics have been demonstrated in this and other laboratories. This work has been extended using dogs anesthetized with morphine-chlorobutanol. In addition to procaine and its analogues, naturally occurring and synthetic parasympatholytic agents were used. These drugs were administered intravenously by slow infusion at a constant rate and continuous recordings were obtained of blood pressure, respiration and electrocardiographic changes. Both intact and severed vagi were stimulated electrically with an inductorium and pharmacologically with parasympathomimetic drugs. Atropine initially blocked the left vagus to electrical stimula-

tion at a dosage of approximately 6.25 gamma/kg., whereas, procaine produced a similar block at 12.0 mg/kg. At these dosages, the cardiac changes induced by parasympathomimetic drugs were also blocked. It was observed that at the point where both vagi were blocked by atropine, control studies revealed insignificant changes in physiological functions. Acetylcholine in doses up to 16.0 gamma/kg. failed to elicit a response as did the electrical stimulation of the peripheral stumps of the vagi. Further increase in the dose of acetylcholine produced the classical 'nicotinic' effect, while increase of current strength produced no quantitative alterations. On the other hand, procaine produced an increase in the respiratory rate and elevation of the T wave, during control studies. The 'muscarinic' action of acetylcholine was easily demonstrated if the blocking agent was a synthetic local anesthetic or the analogues thereof. The exact mechanism of action of these diverse classes of drugs with parasympatholytic activity, especially when the exact mode of hypotension is considered, constitute another approach to this problem.

An empirical comparison of intra-test and inter-test error of the LD_{50}. LYLE D. CALVIN AND HOMER B. FREESE (introduced by W. E. HAMBOURGER). *Div. of Biological Research, G. D. Searle & Co., Chicago, Ill.*

The error assigned to an LD_{50} should reflect the inter-test variation which would be encountered if a large number of determinations of the LD_{50} were made in standard laboratory practice. The objective of these experiments was to compare intra-test error, i.e. the standard deviation of the LD_{50} as calculated from a single determination, with inter-test error. Eight tests were run on each of 6 compounds to determine LD_{50}'s in mice. Standard deviations of the LD_{50}'s were calculated *1*) by the usual formula, as given by Bliss, for a single test determination and *2*) from the series of 8 LD_{50}'s on each compound. The test dates, personnel and compound solutions differed from test to test. In spite of the variability of these factors, the results for all compounds showed that the inter-test error was not significantly greater than the error obtained from a single determination. It may be concluded that with the existing conditions, the standard deviation of a single determination satisfactorily estimated the appropriate error, i.e. the inter-test error.

Disruption of external respiration by eserine and its restoration. C. A. DE CANDOLE (introduced by M. K. MCPHAIL). *Suffield Experimental Station, Alberta, Canada*

Spirometric and other evidence is presented showing that, in the monkey given eserine intravenously, the proximate cause of death is failure of pulmonary ventilation. From studies of how failure comes about, it is clear that in this species, of the 3 or more factors known to be involved (disorganization of central control, bronchospasm, neuromuscular block) the first is the important one. Minimal doses upset the rhythm of the respiratory movements, and total and permanent arrest of breathing result from doses which produce little or no evidence of either bronchospasm or neuromuscular block. Pneumograms show features which appear characteristic. Although the great antidotal value of atropine given subsequent to eserine is well known, there was no good evidence relating this to any action upon the central control of respiration until the work of Douglas and Matthews (*J. Physiol.* 114, 31, 1951). Relaxation of the spastic bronchi by atropine will, of course, remove one element hampering ventilation, but much more important in this species at least is its ability to restore regularity and power to the enfeebled, disorganized breathing movements, and its property of making it possible for breathing to be restarted after it has once stopped, both of these being attributable to actions on the central mechanism. Depending on the circumstances in which it is given, at least three entirely separate responses to the injection of atropine can be recognized, and their interpretation raises fundamental issues which appear capable of shedding new light on the central and reflex control of respiration.

Methyl donor formed enzymatically from L-methionine and adenosinetriphosphate. G. L. CANTONI. *Dept. of Pharmacology, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

A compound capable of transferring its methyl group in the absence of energy-rich phosphate is formed enzymatically by reaction 1 (L-methionine + ATP $\xrightarrow{GSH,\ Mg^{++}}$ 'active methionine' + inorganic phosphate) catalyzed by the methionine-activating enzyme (CANTONI, *J. Biol. Chem.* 189: 745, 1951). 'Active methionine' has been isolated from the reaction mixture by a procedure involving removal of Mg^{++}, precipitation of organic and inorganic phosphates with barium and alcohol and separation from residual methionine by paper chromatography. After elution from paper, the intermediate will methylate nicotinamide or guanidoacetate in the presence of the specific methylpherases. Using S^{35}-methionine it was discovered that the intermediate isolated as above *a*) contains sulfur, but no phosphorus; *b*) has an ultraviolet absorption spectrum resembling that of a 9-substituted adenine compound; *c*) contains pentose as determined by the Bialorcinol reaction. After treatment with HNO_2 the compound had a different R_f; its maximum extinction shifted to 250 mμ., but it still contained sulfur and pentose.

The significance of these and related findings and their implications to the problem of the chemical nature of the methyl donor formed enzymatically will be discussed.

Electrocardiographic changes during endotracheal intubation. R. T. CAPPS, A. M. MACKAY* AND O. S. ORTH. *Depts. of Pharmacology and Anesthesiology, Univ. of Wisconsin Med. School, Madison.*

As part of a study of electrocardiographic changes during clinical anesthesia, particular attention has been directed to the possibility of alterations of cardiac rhythm as the endotracheal tube was inserted. Control electrocardiograms of the conventional limb leads I, II and III were made using a direct-writing electrocardiograph before anesthesia was started, and the beam, usually lead II, was then observed continuously throughout anesthesia. At the time of intubation, electrocardiograms were made continuously during the insertion, or attempted insertion, of the tube into the trachea. The beam was closely observed and electrocardiograms made during the period immediately following intubation to determine if electrocardiographic changes occurred. It seems that there is an increase in number and severity of irregularities for the agents used, in the following order: intravenous barbiturates; barbiturates with curare-like drugs; ether-N_2O; cyclopropane-N_2O, and chloroform.

Determination of activity of arterial adenosine triphosphatase. C. JELLEFF CARR, FEDERICK K. BELL* AND JOHN C. KRANTZ, JR. *Dept. of Pharmacology, Univ. of Maryland School of Medicine, Baltimore, Md.*

The methods available for the determination of ATPase activity of tissues of the vascular system where the total amount of enzyme is small and where the quantity of tissue is very limited, are not satisfactory. A method for the determination of the ATPase activity of aortic arterial tissue based on the micro determination of phosphorus as magnesium ammonium phosphate and its subsequent colorimetric estimation has been developed. The results obtained by this method in an examination of the aortic tissues of the rabbit, rat, turtle, dog, chicken and frog are presented. An examination of the ATPase activity of other segments of the vascular system of the dog has been made. The method has yielded satisfactory results in the determination of ATPase activity of other tissues, e.g. brain and heart.

Excitement stage of barbiturate action. RALPH J. CAZORT. *Dept. of Pharmacology, Meharry Med. College, Nashville, Tenn.*

Adult white rats were given 10 daily doses of barbiturate per os and records made of the degree of excitement, sedation and narcosis, particular attention being paid to the excitement obtained after repeated administration. Evipal Sodium and Seconal Sodium were used. With doses of Evipal Sodium (80 mg/100 gm. body weight) decreases in the sleeping time became evident on the 3rd day. At this time one third of the experimental animals fell asleep, the sleep lasting about half the time of that occurring on the first day. Excitement in those animals not falling asleep was apparent, by observation and by graphic record. On the 4th and 5th day, all animals remained awake; half showed sedation and half were excited. The syndrome of excitement lasted 5–12 minutes; such excitement did not occur in the controls. By the 10th day, excitement as well as sedation had largely disappeared; full sensitivity returned, however, after a week of rest. With Evipal Sodium (40 mg./100 gms. B.W.) there was only sedation, less pronounced on the 3rd day. Mild excitement developed on the 5th day, and was evident on all subsequent days. Another series of animals given Seconal Sodium (60 mg/100 gm. B.W.) showed reductions in sleeping times after the 4th day. In this series, excitement was neither consistent nor marked. Graphic records made of the excitement periods appear to confirm the observations; analysis of the records is still in progress.

Distribution and excretion of radioactivity in rats injected with C^{14} labeled Priscoline-HCl. B. CENTURY,* L. E. ELLINWOOD,* J. D. KOHLI* AND J. M. COON. *Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.*

Radioactive Priscoline labeled with C^{14} at the alkyl carbon linkage and having an activity of 1.0 μc/mg. was made available to us for this study (Ciba). Urine and stools were collected separately from 12 white rats injected intravenously with 7.95 mg/kg. Animals were sacrificed at 1, 2, 5 and 12 hours after injection. Two rats were injected intraperitoneally and two subcutaneously with 15 mg/kg. and sacrificed after 24 hours. Five animals were given 15 mg/kg. and one 8 mg/kg. intraperitoneally and sacrificed after 48 hours. All the tissues studied except brain showed the ability to concentrate the radioactivity from the blood. Liver showed the greatest uptake and slowest release of radioactivity, its concentration being 20 times that of blood in 5 hours. Kidney, lung and adrenal concentrations were 9, 3.6 and 2.6 times that of the blood respectively, while the brain concentration was less than one-third that of the blood. Carcass and muscle concentrations were about twice the blood levels. In one hour 38.7% of the injected radioactivity was recovered in the urine, and in 12 hours 90.7% was recovered. No further recoveries were obtained after 48 hours. Stool recoveries were less than 1.5% after 48 hours.

An average of 0.21% of the radioactivity was recovered from exhaled air of three animals tested for 24 hours. Paper chromatographic studies have suggested that most, if not all, of the radioactivity in the urine represented unchanged Priscoline.

Comparison of 11-hydroxy cardiac glycosides and aglycones with cortisone and compound F. K. K. Chen, Robert C. Anderson*, and Charles L. Rose.* *Lilly Research Lab., Indianapolis, Ind.*

It has been repeatedly suggested that sarmentogenin may serve as an intermediate for the preparation of cortisone or compound F. This prompted us to investigate a group of 11-hydroxy cardiac glycosides and aglycones for cortisone-like action. Sarmentocymarin, ouabain, sarmentogenin, ouabagenin, gamabufagin and gamabufotoxin were studied with cortisone and F as controls. Two pharmacologic methods were adopted—the measuring of liver glycogen deposition and the counting of circulating eosinophiles, both in adrenalectomized mice. All the compounds were made into very dilute alcoholic solutions, and suitable doses were injected subcutaneously. The results with the cardiac substances in both tests were uniformly negative in relatively large doses. On the other hand, both cortisone and F were effective in reducing eosinophiles and increasing liver glycogen.

Effect of tetraethylthiuram disulfide on toxicity of ethanol and acetaldehyde. George P. Child, Malcolm Crump* and Peter Leonard*. *Dept. of Physiology and Pharmacology, Albany Med. College, Albany, N. Y.*

The LD_{50} of ethanol, EtOH, in rats was 7.7 gm/kg., using 50% EtOH. After sensitization with tetraethylthiuram disulfide, TTD, 'Antabuse', the LD_{50} was significantly less, 6.5, 5, 4.4, and 4.1 gm/kg. at TTD dosages of 1.5, 3, 5, and 7.5 gm/kg., respectively. The duration of the alcoholic stupor among the surviving animals was also increased. Since TTD is alleged to produce its sensitization to EtOH by inhibiting its oxidation from proceeding beyond the acetaldehyde, AcH, stage, it was of interest to determine the effect of TTD on the toxicity of AcH. The LD_{50} of AcH was 1.23 gm/kg., using 4% AcH. There was no effect of TTD on either the LD_{50} of AcH or the duration of its effects. To simulate the situation in which man might imbibe EtOH repeatedly while taking TTD, rats were given daily doses of both drugs. In control animals receiving 4.2 gm/kg/day of EtOH there was no cumulative effect. The rats receiving both drugs, however, showed a progressive increase in the toxicity of this daily dose of EtOH. All the animals receiving 50 mg/kg/day of TTD with the test dose of EtOH expired in 6 days. The smallest effective dose of TTD, 2 mg/kg/day, caused a progressive weight loss for the 15 days during which the rats received both drugs. In comparable experiments using AcH in a test dose of 0.7 gm/kg/day there was no cumulative effect.

Antispasmodic activity of a series of benzoyl cyclo-carboxylic acid esters of diethylaminoethanol. Byron B. Clark, Elizabeth Grace,* Ruth Mitchell* and Murray Blair* *Pharmacology Dept., Tufts College Med. School, Boston, Mass.*

The compounds in this series were screened by the Magnus isolated rabbit ileum technique and assayed in comparison with atropine in a 4 square design. The more active compounds were assayed for atropine activity on the cat blood pressure using a continuous infusion of carbaminoylcholine and epinephrine. In addition, the acute toxicity in mice and the stability to esterase breakdown in fresh human serum were determined. The parent compound is the benzoylacetic acid ester of diethylaminoethanol which is inactive, however substitution of saturated cyclic rings on the α carbon confers appreciable activity. The highest activity for the tertiary amines was found for the cyclopropyl ring (N-diethylaminoethyl-1-benzoylcyclopropyl carboxylate); activity decreased in the following order: cyclopentyl, cyclobutyl, and cyclobexyl. The methylbromide quaternary amine analogue of the cyclopropyl compound (2 - ethyl(1 - benzoylcyclopropane - 1 - carboxylate) methyl diethylammonium bromide) had about the same activity on the rabbit ileum, but higher specific atropine-like activity on the cat blood pressure test. Both compounds are moderately stable in human serum, with the tertiary amine being more stable than the quaternary amine. The activities and the toxicities of the compounds are sufficiently favorable to indicate determination of activity in man.

Correlation of psychologic tests and achievement records with speed of visual accommodation in man. Randolph Clements,* John Dale Archer,* Robert G. Brown* and G. A. Emerson. *Univ. of Texas Med. Branch, Galveston.*

Speed of visual accommodation was measured with the tachistoscope devised by C. H. Ferree and G. Rand (*Arch. Ophthalmol.* 15: 1072, 1936). Two groups of subjects, totaling ca. 350, were studied. The 1st comprised sophomore and junior medical students and student nurses; the 2d, inmates of the Texas Prison System. Robertson (*Arch. Ophthalmol.* 14: 82, 1935) notes a correlation with intelligence, within the same age groups; we have confirmed the significant increase in time of accommodation with age. Since results of the 2 groups agreed within close limits, correlation with objective tests of intelligence and achievement were made for each group. For the medical students, M. C. A. T. scores and pre-medical averages

were available; for the prison inmates, the new Stanford Achievement, the quick-scoring Otis test, the Minnesota Multiphasic Personality Inventory and the Lee and Thorpe Occupational Interest test were available. Results indicate that factors of intelligence or training are of minor significance, despite the admittedly considerable importance of immediate memory in estimating speed of visual accommodation with the tachistoscope.

Inhibitory action of steroid hormones on citrate synthesis in vitro. KENNETH W. COCHRAN* AND KENNETH P. DUBOIS. *U. S. Air Force Radiation Lab. and Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.*

Recent investigations in this laboratory showed that the administration of fluoroacetate to female rats caused citrate accumulation in the liver in contrast to the inability of male rats to accumulate citrate following fluoroacetate. Evidence that an inhibition of citrate synthesis by certain steroid hormones might be responsible for this sex difference was obtained from experiments on castrated, adrenalectomized, and x-irradiated rats and from the inhibition of citrate and acetoacetate synthesis by liver homogenates observed *in vitro* with testosterone and desoxycorticosterone. The present investigation was undertaken to obtain further information on the influence of various steroids (Schering Corp.) on citrate synthesis by liver and other tissues. Citrate formation was measured using the system of Pardee and Potter (*J. Biol. Chem.* 178: 241, 1949). The average oxygen consumption and citrate formation uM/50 mg/40 min.) for normal rat tissues were: liver, 7.2 and 3.5; kidney, 20 and 2.5; brain 4.9 and 1. Methyl androstenediol, estrone, cortisone acetate, cholesterol, and ouabain were ineffective as inhibitors while testosterone, desoxycorticosterone, progesterone and diethylstilbesterol effectively inhibited citrate synthesis and oxygen consumption by liver, kidney, and brain with kidney being the most sensitive *in vitro*. Testosterone (200 ug/flask) inhibited oxygen consumption of liver 52% and citrate formation 50%, while the values for kidney were 59% and 50%, and brain 25% and 46%. Desoxycorticosterone (200 ug/flask) inhibited citrate synthesis by liver 74%, kidney 83%, and brain 46%. Progesterone (200 ug/flask) inhibited liver 82%, kidney 99%, and brain 62% and diethylstilbesterol was similarly effective.

Relationship between barbital concentration and antagonism to metrazol. VERSA V. COLE AND H. R. HULPIEU. *Indiana Univ. School of Medicine, Indianapolis.*

Male mice weighing from 20–30 gm. were used as experimental animals. Metrazol, 50 mu/gm. administered to mice by vein killed 20 out of 20 mice. Two minutes after barbital sodium, 300 mu/gm. with a brain concentration of 38 mu/gm. of barbital, 20 out of 20 mice survived the same dose of metrazol. From 30 minutes to 12 hours after barbital the survivors from metrazol varied from 6–8 mice out of 10 injected. At 12 hours no barbital was found in the brain while at 1 hour with the same survival rate the concentration in the brain was 154 mu/gm. Appreciable concentrations of barbital were found at 12 hours in the whole mouse, liver, kidney, muscle and blood. After 16 hours it was not possible to find barbital in these tissues, and yet the survival rate of mice given metrazol from 16–24 hours after barbital was 30%. If the antagonism to metrazol by barbital is a function of the concentration of barbital an extremely low concentration suffices to give protection.

Chronic inhalation toxicity of hydrazine vapors to dogs and rats. CHARLES C. COMSTOCK* AND FRED W. OBERST. *Chemical Corps Med. Labs., Army Chemical Center, Md.*

The chronic inhalation toxicity of hydrazine vapors (concentration, 6 mg/m³) was determined on 2 dogs and 20 rats. One dog and 10 rats served as controls and were kept under the same conditions as the experimental animals. Exposures were made 6 hours each day, 5 days/week, for 31 weeks. One dog lost 16% of his original body weight and the other 29%. There were periods of loss of appetite alternating with periods of normal food consumption throughout the experiment. Beginning with the 11th week the loss of appetite was accompanied by muscle tremors, weakness and vomiting. These signs became more severe with each period of loss of appetite. During the course of the experiment, blood counts and blood chemistry were normal except for an increased stab count and a mild hypoglycemia toward the end of the experiment. The rats continued to gain weight throughout the experiment at the same rate as the controls. Beginning with the 23d week the rats became lethargic. Two died during the 28th week. Autopsies of the dogs and rats showed that tissue alterations were confined chiefly to the lungs. The rats exhibited an interstitial pneumonitis with a few localized areas of alveolar emphysema. The dogs' lungs had alternating patchy areas of emphysema and atelectasis which were sufficiently large to be visible grossly. The musculature of the bronchioles was found to be undergoing hypertrophy.

Excretion and concentration of antibiotics in bile. D. L. COOK*, COLETA A. LAWLER* AND D. M. GREEN. *Div. of Biological Research, G. D. Searle & Co., Chicago, Ill.*

Bile and plasma concentrations were measured in a group of anesthetized dogs following intravenous doses of various antibiotics. Penicillin was

highly concentrated in the bile, its maximum being 28 mM/l. compared to 0.2–0.7 mM/l. for the other antibiotics at the dosages studied. The biliary concentrations of dihydrostreptomycin, terramycin and aureomycin tended to approach maxima relatively independent of their plasma levels, suggesting saturation of hepatic transfer mechanisms. This phenomenon was particularly evident for aureomycin, which reached the same plateau of biliary concentration despite fourfold variations in dose and tenfold variations in plasma concentrations.

Effect of mercurial diuretics on enzymes of kidney. JOHN H. COPENHAVER* AND ANDRES GOTH. *Dept. of Pharmacology, Southwestern Med. School, Univ. of Texas, Dallas.*

Comparative studies have been made of the inhibition of a number of enzymes in rat kidney homogenates by sulfur-linked (Thiomerin) and non-sulfur linked (Mercuzanthin, Mercuhydrin) mercurial diuretics. This work has been carried out under the postulate that since the 2 types of mercurials are of approximate equal effectiveness as diuretics, the affinity of the enzymes involved in salt reabsorption should be of the same order of magnitude for both types of drugs. It is further postulated that such enzymes will be among those having the greatest affinity for the mercurial diuretics. Studies on anaerobic glycolysis, the tricarboxylic acid cycle, various dehydrogenases, transaminase and other enzymes will be reported.

Cardiovascular and central nervous system actions of cassaine and cassaidine. M. DEV. COTTEN*, L. I. GOLDBERG* AND R. P. WALTON. *Depart. of Pharmacology, Med. College of South Carolina, Charleston.*

Cardiovascular effects of cassaine and cassaidine were determined in open-chest dog preparations according to methods previously used in characterization of other Erythrophleum alkaloids (*Federation Proc.* 10: 299, 1951). About 14 experiments were conducted with each alkaloid and a similar additional series with ouabain was included for comparison. In all experiments, after stable levels of response to the initial drug, ouabain was infused to termination. Contractile force, arterial pressure and ECG effects with cassaine and cassaidine were qualitatively similar to those of ouabain. Quantitatively, cassaine produced greater contractile force increments at the dose range of 0.25 cat unit/kg. At other dose levels the distinctions were not significant. There were no differences in persistence of contractile force increments at any of the dose levels. With cassaine and cassaidine, however, the arrhythmias were conspicuously more transient than with ouabain. This feature of transient arrhythmias was also demonstrated by greater supplemental doses of ouabain required to terminate the experiments and by a separate series of repeated injections in cats. In unanesthetised dogs, cassaine produced static ataxia at dose levels of 0.25 CU/kg. i.v. and bizarre convulsive seizures with 0.5 CU/kg.; cassaidine produced ataxia, cataleptic postures and no convulsions with 0.7 cat unit/kg.; cassaidine in doses of 0.5 CU/kg. consistently produced emesis but no important effect on Thiry-Vella fistula tracings. These crystalline alkaloids were prepared by Ruzicka *et al.* and obtained through the courtesy of R. Meier, Basle, Switzerland. Cat unit strengths were found to be approximately the same as reported by Chen *et al.* (*J. Am. Pharmacol. Assoc.*, 27: 9, 1938).

Enzyme inhibition as a cause of sensitization of vessels to the vasoconstrictor action of adrenaline. GEORGE C. COTZIAS, THEODORE BALOURDAS AND VINCENT P. DOLE (introduced by A. E. COHN). *Hosp. of the Rockefeller Inst. for Med. Research and the Dept. of Biology, New York Univ., New York City.*

The Monoamine Oxidase (MAO) of the rat's mesentery was demonstrated to be localized in the walls of the vessels, by staining the aldehyde produced during the oxidative deamination of Tyramine. The mesentery's MAO activity was measured by the rate of deamination of 3×10^{-3}M Tyramine at 20°C. Benadryl and indole acetic acid were demonstrated to be *in vitro* inhibitors of MAO. Similarly, inhibition of the specific activity of MAO of the rat's mesentery after *in vivo* intravenous injection of the inhibitors was found. The degree of inhibition was proportional to the amount of inhibitor injected. That the inhibitor-enzyme combination was not caused by the manipulations involved was shown by the failure of the animals' blood to cause appreciable inhibition of MAO, 15 minutes after injection. Local or intravenous administration of the inhibitors had no observable effect on the rat's mesoappendiceal vessels. The intravenous injections were followed by a dramatic increase in the sensitivity of the mesoappendiceal vessels to locally applied adrenaline. The adrenaline threshold decreased from 4×10^{-6} to 1×10^{-9}. The sensitization appeared 5–15 minutes after injection, became maximal after 20–30 minutes and disappeared after 90 minutes. It was proportional to the amount of injected inhibitor. The view that this phenomenon is probably restricted to amines which are MAO substrates was supported by the failure of the inhibitors to cause an increase in the sensitivity to ephedrine.

Action of salicylic acid on the adrenal-pituitary system. GEORG CRONHEIM, J. STANTON KING, JR.* AND NELTA HYDER.* *Research Dept., The S. E. Massengill Co., Bristol, Tenn.*

At a previous meeting (*J. Pharmacol. & Exper. Therap.*, 103: 341, 1951) experimental data were presented indicating an action of salicylic acid (SAL) on the adrenal-pituitary system. It was concluded that this drug has a specific effect which results in an increased output of ACTH by the pituitary. The experiments were extended to include prolonged exposures to repeated doses of SAL, as well as combinations of the drug with various aminoacids and with intermediates of carbohydrate metabolism (citric, gluconic, glucuronic and succinic acid). In most instances the combinations produced the same results as SAL alone, thus providing additional evidence for the specificity of the observed phenomena. However, one case of potentiation of the SAL effect by an aminoacid was observed. Blood-level determinations indicated no change in SAL absorption. In order to study the mechanism of the SAL action the influence of a barbiturate was investigated. In normal rats, deep anesthesia with pentobarbital sodium significantly reduced the depletion of adrenal ascorbic acid following SAL administration, but it did not abolish it entirely. There was also evidence that the depth of anesthesia was of considerable importance. SAL blood-levels in the pentobarbital-treated animals were the same as in normal controls. Pretreatment with atropine did not influence the reduction in adrenal ascorbic acid produced by SAL.

Cerebral circulatory and metabolic changes following intramuscular hexamethonium bromide (C-6) in subjects with severe hypertensive retinopathy. C. W. CRUMPTON*, Q. R. MURPHY,* J. J. WHITMORE*, R. T. CAPPS AND O. S. ORTH. *Depts. of Medicine, Pharmacology, and Physiology, Univ. of Wisconsin Med. School, Madison.*

Cerebral hemodynamics and oxygen metabolism (nitrous oxide method) were studied in 6 hypertensive subjects in the supine position before and 1 hour after intramuscular hexamethonium bromide (0.8–1.2 mg/kg.). All subjects exhibited a grade III–IV degree of hypertensive retinopathy (Keith-Wagener classification). Intramuscular C-6 resulted in a 29% reduction in mean arterial blood pressure ($p < .02$) and a 30% decrease in cerebral vascular resistance ($p < .02$) without essential change in cerebral blood flow (56 and 57 cc/100 gm/min.). The increase in oxygen uptake of the brain from 3.6–4.0 cc/100 gm/min. was not statistically significant in 6 patients. A significant decrease in internal jugular venous oxygen saturation ($p < .01$) was observed during the period of blood pressure reduction. Under the experimental conditions described, the equal reduction in mean arterial blood pressure and cerebral vascular resistance without change in the volume of blood flow through the brain indicates a favorable therapeutic effect of hexamethonium bromide upon cerebral hemodynamics in essential hypertension. However, the significant reduction in internal jugular venous oxygen saturation suggests that a satisfactory cerebral metabolic adjustment to the decrease in blood pressure had not occurred 1 hour following drug administration.

Interrelation of drugs, ions and metabolism in uterine muscular activity. EDWIN E. DANIEL (introduced by LOUIS S. GOODMAN). *Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City.*

Uterine responses to sympathomimetics, parasympathomimetics, histamine and pitressin, and effects of blocking agents have been studied *in vitro*. Responsiveness to sympathomimetics, as to other agents producing contraction, is correlated with the potentiality of the uterus for spontaneous activity. Tonus (contracture), rather than inactivity, causes the uteri of non-pregnant cats and guinea pigs to respond to sympathomimetics by relaxation rather than by contraction. If contracture is relaxed, responses to all contraction-producing agents are restored or improved. Progesterone pretreatment of non-pregnant cats reduces the tendency of the uterus to undergo contracture. N-isopropyl-norepinephrine, epinephrine, histamine and sometimes norepinephrine, after they are administered and washed out, temporarily depress spontaneous activity and responsiveness to contraction-producing agents. Blockade of their excitatory actions enhances their depressant actions. Ringer's solution which has been in contact with active strips does not restore activity to depressed strips. Moderate dosages of pitressin, acetylcholine and methacholine promote spontaneous activity and responsiveness and antagonize the depression produced by sympathomimetics and histamine; higher doses produce post-wash depression. Anoxia, iodoacetate or prolonged deprivation of exogenous glucose depresses both spontaneous activity and responsiveness to contraction-producing agents and potentiates and prolongs the post-wash depression produced by such agents. Calcium deficiency (during recovery only) prolongs whereas potassium deficiency shortens the depressant effects of contraction-producing agents. Recovery in the absence of both calcium and potassium is very slow. It is postulated that autonomic agents have important actions on an energy-requiring system necessary for activity of uterine smooth muscle different from the receptor systems. This system may be the active transport of cations.

L-isomethadone and morphine analgesia: potentiation by dihydrogenated ergot alkaloids in the rat. NORMAN A. DAVID AND HERBERT J. SEM-

LER*. *Dept. of Pharmacology, Univ. of Oregon Med. School, Portland.*

Small doses of DHE #45, DHO #180 and CCK #179 (Sandoz) ranging from 0.2–0.6 mg/kg. were found to block the hyperglycemia caused by single doses of 15 mg/kg. of morphine sulfate or 5 mg/kg. of either L-isomethadone, DL-methadone or L-methadone in rabbits (N. M. PHATAK, AND T. T. NITTA, Fall Meeting, Amer. Soc. Pharmacology, Oct. 15–17, 1951, Omaha, Neb.). These results prompted us to study the effects of combinations of these drugs on analgesic activity using the D' Amour-Smith technic with Sprague-Dawley rats weighing from 150–200 grams. The dihydrogenated ergot alkaloids were administered subcutaneously 30 minutes before the i.p. injection of the analgesic drug to groups of from 5–8 rats. For each trial a control group was injected i.p. with the same dose of the analgesic drug alone. No significant analgesic action was noted for the dihydrogenated ergot alkaloids when administered i.p. alone in the dose range we used. Results indicate that preliminary administration of such drugs as DHE 45, DHO 180 and CCK 179 before administration of an analgesic drug significantly enhances analgesic action compared to the effects obtained from the same dose of the analgesic drug given alone; also, potentiation of analgesia appears greater when small doses of the order of 0.03 mg/kg. to 0.10 mg/kg. of either CCK #179 or DHO #180 rather than larger doses are used. With the i.p. injection of 6 mg/kg. of morphine sulfate alone, the mean percentage increase in reaction time was 86.9% while in the group given a preliminary subcutaneous administration of 0.03 mg/kg. of CCK #179, this result rose to 161.9%.

Metabolite of heptachlor, its analysis, storage and toxicity. BERNARD DAVIDOW AND JACK RADOMSKI (introduced by A. J. LEHMAN). *Div. of Pharmacology, Food and Drug Admin., Federal Security Agency, Washington, D. C.*

An analytical method has been developed for the determination in biological tissue of heptachlor epoxide, reported elsewhere to be a metabolite of the chlorinated insecticide called heptachlor. Ether extracts were dissolved in hexane and extracted with 95% methanol. The methanol fraction was diluted with water and extracted with hexane. The hexane was concentrated and passed through a column of alumina. The metabolite was eluted with 10% benzene in hexane and concentrated to 1 ml. Three ml. of a reagent consisting of 2 parts methanolic potassium hydroxide and 1 part diethanolamine were added and the mixture heated for 10 minutes at 91°C. From readings taken at wavelengths 390, 415, 440 millimicrons, the concentration of the metabolite was calculated. Results with both rats and dogs demonstrate that the metabolite is stored principally in the adipose tissue. Smaller amounts are also found in liver, muscle and kidney. Female rats fed a diet containing 30 ppm. of heptachlor for 2 weeks contained 165 ppm. of the metabolite in the fat. Male rats under similar conditions averaged 61 ppm. Two dogs receiving 3 mg/kg. for 95 days and 1 mg/kg. of heptachlor for 495 days had approximately 500 ppm. of the metabolite in fat. When injected intravenously into male mice, the metabolite was between 2 and 4 times more toxic than heptachlor. The toxicity of both compounds to the common house fly was of the same order.

Effect of various stigmine type compounds on enzymatic hydrolysis of procaine in plasma. DAVID L. DAVIS AND FRANCIS F. FOLDES (introduced by L. O. RANDALL). *Dept. of Anesthesia, Mercy Hospital, and Department of Surgery, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.*

The inhibitory effect of neostigmine and RO2-683 on the enzymatic hydrolysis of procaine in plasma was reported previously by Aven and Foldes (*Federation Proc.* 10: 295, 1951). The inhibitory effect of several other stigmine type compounds was since investigated. Various amounts of the compound to be studied, dissolved in 0.2 ml. of distilled water, was added to 1.6 ml. of heparinized plasma samples and was incubated at 37°C for 5 minutes. Following this 0.2 ml. of a 1.0 mg/ml. procaine solution was added and the incubation continued for 10 minutes. The substrate concentration was 0.1 mg/ml. in all experiments. The inhibitor concentration varied from 1:10,000 to 1:100,000,000. A control sample to which no inhibitor was added was included in each series. The inhibitory effect was expressed as:

$$\% \text{ inhibition} = 100 - \frac{\text{procaine hydrolyzed with inhibitor}}{\text{procaine hydrolyzed without inhibitor}} \times 100$$

With two exceptions the compounds investigated showed in dilutions up to 1:1,000,000 an inhibitory effect of about 90%. The inhibitory effect of dilutions between 1:1,000,000 and 1:20,000,000 was of the order of 70%. In 1:100,000,000 dilution the inhibition was about 10% to 20%.

Effect of adrenalectomy and replacement therapy on ascites formation in dogs. JAMES O. DAVIS, DAVID S. HOWELL AND JAMES L. SOUTHWORTH (introduced by JAMES H. BAXTER). *Nat. Heart Inst., Nat. Insts. of Health, Bethesda, Md., and U. S. Public Health Service Hosp., Baltimore, Md.*

Following right adrenalectomy, ascites was produced by constriction of the thoracic inferior vena cava. Renal function and metabolic balances

were studied during a 3-week control interval and for 7 months after left adrenalectomy. In the presence of replacement therapy, ascites continued to accumulate. Renal function and electrolyte excretion remained unchanged except for the disappearance of the low Na and high K pattern of fecal electrolyte excretion which is characteristic of experimental ascites. Discontinuation of hormone therapy resulted in a diuresis; the general appearance and vigor of the animals was excellent until adrenal insufficiency supervened after an interval of 10–14 days. The DCA requirement for maintenance of adrenalectomized dogs with ascites and after removal of the caval ligature and disappearance of ascites was essentially the same (.5 mg/day). However, additional DCA was necessary for the accumulation of ascites. Following removal of the caval ligature, ascites disappeared despite the administration of 3 mg/day of DCA. GFR was markedly elevated above the control level during this diuresis. It is concluded that adequate adrenocortical function or replacement therapy is necessary for the formation of ascites on a normal salt intake.

Paraphenylene-diamine anemia in rats. JOHN EMERSON DAVIS. *Univ. of Texas College of Pharmacy, Austin.*

Ten white albino rats were given single subcutaneous injections of paraphenylene-diamine dihydrochloride ranging from 50–80 mg/kg. of body weight. Higher doses proved to be fatal within 5 hours. This procedure caused the development of anemia within 3 days in all rats, without the appearance of any detectable localized edema such as has been found in other species. Erythrocyte numbers reached maximum reductions ranging from 17–40%, with an average of 27%, and hemoglobin percentages were reduced, in general, proportionately. The anemia persisted for periods ranging from 15–30 days.

Antidiuretic action of L-epinephrine. EARL H. DEARBORN AND LOUIS A. LASAGNA.* *Dept. of Pharmacology and Experimental Therapeutics, Johns Hopkins Univ. School of Medicine, Baltimore, Md.*

The effect of L-epinephrine upon a water diuresis has been studied. The diuresis was produced in trained female dogs by administration of 40 ml. water/kg. body weight by stomach tube. The L-epinephrine was injected intravenously, as a single injection lasting 2–4 seconds, when the urine flow had attained a rate of approximately 3 ml/minute. Small doses of L-epinephrine produced simultaneous transient decreases in urine flow and creatinine clearance. Larger doses of L-epinephrine produced a similar transient decrease in creatinine clearance and a prolonged decrease in urine flow with normal creatinine clearance.

Cross tolerance to hyperglycemic effects of analgesic drugs. D. DE BOER, E. F. KEITH* AND L. WALTS.* *Dept. of Pharmacology, St. Louis Univ. School of Medicine, St. Louis, Mo.*

Kimura *et al.* (*J. Pharmacol.* 101: 1951) reported hyperglycemic responses following morphine 5, methadone 2, Nisentil 5, and Dromoran 2 mg/kg. and tolerance to this effect following daily intravenous injections for 9 weeks (*Federation Proc.* 10: 1951). Rabbits were given daily intravenous injections of these drugs for 20 weeks and weekly blood sugar determinations made on venous blood using the Somogyi-Nelson colorimetric method. After 9 weeks, the blood sugar determination followed administration of the same drug to all rabbits including saline controls one day of each week. Maximal blood sugar in mg% obtained following administration of morphine to the respective groups of animals was: morphine 87, methadone and Nisentil 96, Dromoran 98, saline 132. Following methadone: methadone 101, morphine 96, Nisentil 108, Dromoran 119, saline 121. Following Dromoran: Dromoran 102, morphine 81, methadone 96, Nisentil 89, saline 129. Following Nisentil: Nisentil 86, morphine 96, methadone 104, Dromoran 119, saline 127. Morphine produced the greatest tolerance and caused the least changes in blood sugar when injected into animals tolerant to other drugs. Dromoran, when injected into animals tolerant to morphine, methadone, or Nisentil, produced greater hyperglycemic changes than any of the other drugs used.

Glucuronic acid lactone, a growth factor in rats. WM. B. DEICHMANN, JOHN J. CLEMMER AND CHAS. DOWNING. *Div. of Pharmacology, Dept. of Physiology and Pharmacology, Albany Med. College, Albany, N. Y.*

The effect of glucuronic acid lactone on body weight of albino rats was investigated over a period of 52 weeks. The material was added to the drinking water. Each of one group of animals (15 males and 15 females, av. wt. 95 gm.) ingested per day approximately 54 mg. of glucuronic acid lactone, equivalent to approximately 0.03 of an LD_{50}. Each animal of a similar group ingested approximately 108 mg/day equivalent to 0.06 of an LD_{50}. Thirty control animals received uncontaminated water. Signs of glucuronic acid intoxication (*Ind. Med. & Surg.* 20: 417, 1951) were not observed but 5 control and 6 experimental animals died during the experiment. The causes were unrelated to the ingestion of glucuronic acid. All experimental animals gained weight more rapidly than the controls. This became particularly apparent after 15 weeks of feeding, it was more pronounced in males than in females. At the end of the year the male controls weighed an average of 389 gm., males ingesting daily 0.03 of an LD_{50} weighed an average

of 472 mg.; males ingesting 0.06 of an LD_{50} weighed an average of 466 gm. Similar weights for the females were 243 gm. for the control group and 264 and 257 gm. for each of the experimental groups, respectively. All animals were sacrificed, significant gross or histopathological changes were not observed. The observations made support earlier conclusions of Miller, Siehrs and Brazda (*Proc. Soc. Exper. Biol. & Med.* 30: 636, 1932) who noted that glucuronic acid represents a 'growth factor' when added to the diet of guinea pigs. This study appears to indicate that administered glucuronic acid lactone supports growth not only in a species who derives most of this acid from an exogenous source (guinea pig) but also in a species in whom glucuronic acid is primarily of endogenous origin (rat) (*Arch. of Biochem.* 7: 401, 1945). (This investigation was supported by grants from the United States Public Health Service.)

Method of measuring mild analgesia in man. G. A. Deneau, and C. W. Gowdey. *Dept. of Pharmacology, Univ. of Western Ontario Med. School, Ontario, Canada.*

A method for measuring mild analgesia in human subjects has been developed. The method involves the production of a sharp pain in the calf muscles by the application of a steadily increasing pressure. The subject is seated in a chair, a sphygmomanometer cuff is placed around the calf, and its position marked on the leg. With the leg relaxed, the cuff is inflated as rapidly as will permit the operator to read the pressure on a mercury manometer. When the sensation changes from one of pressure to pain, the manometer is read and the pressure immediately released. The average value of 3 readings is taken as the pain threshold at any particular time. When new subjects are used, 2 preliminary trials are made at half-hour intervals before the normal pain threshold is determined. This training is sufficient because of the acuteness of the pain. Measurable, statistically-significant analgesia was produced by acetylsalicylic acid and by codeine and meperidine. The reliability and limitations of the test will be discussed.

Effect of cortisone and 21-acetoxypregnenolone on experimental arthritis. Alphonse Dervinis,* Joseph Seifter and William E. Ehrich. *Wyeth Inst. of Applied Biochemistry, Wyeth Inc., Philadelphia General Hosp., and the Graduate School of Medicine, Univ. of Pennsylvania, Philadelphia.*

Daily injection of 100 TRU of hyaluronidase for 8 consecutive days into the knee joint of rats resulted in a mild to marked proliferative response of the fibroblasts and endothelial cells of the synovia. This was associated with mild leukocytic infiltration and exudation of fibrin into the capsular space. Some rats also developed a few focal granulomas consisting of undifferentiated mesenchymal cells. This was associated with or preceded by fibrinoid degeneration. Both cortisone and 21-acetoxypregnenolone had a beneficial effect on this type of arthritis, cortisone being far more effective, particularly in suppressing the fibrinous exudation into the synovial cavity.

Nutritional value of intravenous tapioca dextrin. L. E. Detrick*, A. E. Lewis*, Bonnie Rhodes* and Thomas J. Haley. *Atomic Energy Project, Univ. of California School of Medicine, Los Angeles.*

Dutch strain rabbits of both sexes were fed intravenously through an indwelling plastic catheter with a balanced nutritional solution containing amino acid hydrolysate, B-complex vitamins, and tapioca dextrin. This mixture resulted in a body weight loss of approximately 19% whereas when 10% glucose was substituted for the dextrin, the weight loss was only 15%. Control animals starved for the same time interval, 11 days, lost 30% and controls injected with the solution containing no carbohydrate lost 22% of their weight. These results indicate that tapioca dextrin is not a good source of available carbohydrate when administered intravenously. However, when fed orally the material can be utilized. When the total daily volume was injected at a rate of 25 ml/hour in two 3-hour periods with a 1-hour interval between injections, 150 ml. daily doses were well tolerated by 2-kg. rabbits. Reversible damage to the kidneys could be demonstrated histologically in those animals which were allowed a 14- to 28-day recovery period. The kidney lesions observed in sacrificed animals were generalized swelling and pallor of the tubular epithelium with no involvement of the glomeruli or collection ducts. No foam cells were observed in the reticulo-endothelial system but the liver was entirely depleted of glycogen. There was no fatty infiltration of the liver. (Based on work performed under Contract No. AT-04-1-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.)

Effect of alloxan administration on liver DNA (desoxyribose nucleic acid). H. F. Diermeier*, H. S. DiStefano* and A. D. Bass. *Depts. of Pharmacology and Anatomy, New York State College of Medicine, Syracuse, N. Y.*

The effect of alloxan administration on liver nucleoproteins has been investigated. Diermeire *et al.* (*P.S.E.B.M.* 77: 769, 1951) showed that the DNA of parenchymal cells of the liver was increased in rats made diabetic with alloxan. It was of interest to determine whether this change was due to alloxan or to the diabetic state. The results previously reported were confirmed. Rats diabetic as a result of depancreatization showed no alteration in the DNA content per nucleus. Alloxan

(400 mg/kg.) was administered intraperitoneally to rabbits. Administered by this route alloxan does not produced diabetes. The livers were examined for nucleoprotein changes. An increase in DNA was observed in the treated animals. Alloxan (160 mg/kg.) was administered intravenously to chickens. As expected blood glucose values remained in the normal range. There was no alteration in the amount of DNA in the treated animals. It has therefore been demonstrated that in the rat diabetes does not cause an increase of DNA in the liver. When alloxan is given intraperitoneally liver DNA is increased in the absence of diabetes. With this route of administration the alloxan is presumably largely destroyed by the liver so that ineffective amounts reach the pancreas. In the chicken the beta cells of the pancreas are relatively insensitive to alloxan, a phenomenon which is apparently shared by the liver.

Effect of some succinic acid analogues on succinoxidase activity. E. V. DIETRICH,* A. L. FISHER,* J. P. LONG* AND R. M. FEATHERSTONE. *Dept. of Pharmacology, State Univ. of Iowa, College of Medicine, Iowa City.*

A series of 34 succinic acid analogues were tested for their activities as substrates or inhibitors of pigeon breast succinoxidase by standard manometric techniques. These compounds were made available by F. W. Schueler and C. Hanna, of this laboratory. One compound, β-(p-benyzlbenzoyl)-propionic acid, had one-half the activity of succinic acid as substrate. Several others, including β-(p-phenoxybenzoyl)-propionic acid, which is closely related to the above compound having substrate activity, were one tenth to one fifth as active as malonic acid in inhibiting this succinoxidase preparation. Also included were the phthalic acids, of which only terephthalic acid was inhibitory. Other compounds showing inhibitory action were β-(mercaptobenzthiazol)-propionic acid, disalicyl succinate, and 5-thio(keto)phenyl acetic acid. The structure-activity relationships of the succinic acid analogues as they affect or fail to affect the activity of succinoxidase and the character of the inhibitions noted will be discussed. (This study has been aided by a United States Public Health Research Grant.)

Pharmacological aspects of phenylbutazone (Butazolidin T.M.), a new antirheumatic. R. DOMENJOZ. (introduced by Charles M. GRUBER.) *Dept. of Pharmacological Research, J. R. Geigy Ltd., Basle, Switzerland and Jefferson Med. College, Philadelphia, Pa.*

Phenylbutazone (Butazolidin T.M.) has shown in clinical trials a definite therapeutic effect in rheumatism and associated disorders. In therapeutic application in man there is besides a marked analgesic and antipyretic effect a definite anti-inflammatory action. These therapeutic effects may, at least in part, be based on the following demonstrated pharmacologic actions: *a*) Anti-inflammatory effect (inhibition, partial or total, of U.V. erythema of guinea pigs; U.V. dermatitis of rats; dermatitis caused in mice and rabbits by croton oil; chemosis in rabbits by ol. sinapsis; edema of rats caused by egg albumin: inflammation by formaldehyde in rats; allergic arthritis by serum in rabbits); *b*) decrease of capillary permeability to colloidal dyestuffs; *c*) antihistaminic effect (isolated gut of guinea pig, detoxification of histamine by guinea pigs, histamine wheal, rabbits ear after Krawkow-Pissemski); *d*) antipyretic action (inhibition of *B. coli* fever in rabbits); *e*) analgesic effect (elevation of threshold to electrical stimulation of the dental pulp of rabbits and dogs). The retarding effect on elimination of sulfonamides and PASA, measurable in animal experiments, may be of additional therapeutic importance.

Influence of certain fluorinated fatty acids on the accumulation of *p*-amino-hippurate by the rabbit kidney slice. ABEL M. DOMINGUEZ* AND F. E. SHIDEMAN. *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.*

Utilizing the method described by Cross and Taggart (*Am. J. Physiol.* 161: 181, 1950) the effects of fluoride (F), monofluoroacetic (FA), 4-fluorobutanoic and 6-fluorohexanoic (FH) acids on the accumulation of *p*-aminohippuric acid (PAH) and oxygen consumption of renal cortical slices of the albino rabbit were determined. FB and FH were found to be about 500 times as potent as FA in inhibiting PAH accumulation. These two fluorinated fatty acids were capable of producing 80% inhibition of PAH uptake by the renal slice at a concentration of 2×10^{-5} M. To obtain the same degree of inhibition with FA a concentration of 1×10^{-2} M was required. The possibility that this inhibition might be due to the liberation of F from the fluorinated compounds could not be disregarded. Molar concentrations of F the same as those of FB and FH, which produced complete inhibition of PAH accumulation, had no significant effect on this function of the renal slice nor upon its oxygen consumption. However, when the F concentration was increased to 5×10^{-3} M, at which molarity FA produces inhibition of PAH uptake by the slice, accumulation of PAH was suppressed about 60% with little effect on oxygen consumption being noted. All concentrations of FB and FH having an inhibitory effect on PAH accumulation decreased oxygen uptake 35% to 53%. However, concentrations (3×10^{-3} M) of these compounds which completely suppressed PAH uptake inhibited oxygen consumption to no greater degree. The effects of FA on oxygen con-

sumption were essentially the same except that with this compound significant inhibition of PAH accumulation by the slice could be obtained with concentrations of FA which had no significant effect on oxygen uptake.

Effects of x-irradiation on *Citellus tridecemlineatus*. JOHN DOULL,* DONALD F. PETERSEN* AND KENNETH P. DUBOIS. *U. S. Air Force Radiation Lab. and Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.*

The increase in survival time of frogs kept at low temperatures following exposure to x-irradiation (H. M. PATT and M. N. SWIFT. *Am. J. Physiol.* 155:388, 1948) suggested that a simiar phenomenon might be observed in hibernating mammals. To test this possibility we undertook studies on the ground squirrel (*Citellus tridecemlineatus*) which serves as an ideal animal for investigating the influence of various toxicants on a hibernating species. Hibernation can be readily induced throughout the year in this species by exposure to temperatures of 5°C. for 24–48 hours, and when the poikilothermic state is attained these animals return to the homeothermic state less frequently than most other hibernators. Non-hibernating ground squirrels exposed to 800 r of x-irradiation suffered a 100% mortality within 10 days. In hibernating ground squirrels given 800 r and maintained at 5°C. thereafter, no deaths occurred within 30 days. When animals from this group were returned to room temperature 2 weeks or 4 weeks after irradiation, they developed the typical radiation syndrome and died within 10 days. Non-hibernating ground squirrels placed at 5°C. after 800 r failed to hibernate and died within 10 days. Loss in body weight in irradiated hibernating ground squirrels was similar to that in non-irradiated hibernating animals. Animals irradiated while in the non-hibernating state exhibited greater weight losses during the same period. Similar effects have been observed in the marmot by Smith and Grenan (*Science* 113: 686, 1951). Studies on the cellular metabolism of hibernating and non-hibernating ground squirrels before and following x-irradiation have been conducted.

Toxicity of certain surface-active agents to eye mucosa. J. H. DRAIZE AND ELSIE A. KELLEY.* *Div. of Pharmacology, Food and Drug Admin., Federal Security Agency, Washington, D. C.*

Synthetic surface-active agents used in drugs and cosmetic preparations have led to incidents of injury. Most synthetic surface-active agents are not exact chemical entities but are condensation products characterized by general empirical formulae. Such agents are designated as anionic cationic and nonionic depending on chemical structure. All three types of surface-active agents are used for topical drug and cosmetic use. Their extensive use in shampoo, bath and hair waving preparations leads to occasional accidental introduction of these agents into the eyes which may produce corneal opacity, iritis, pannus, chemosis, severe congestion and erythema of the palpebral and bulbar mucosa with discharge. Fourteen surface-active agents (4 anionic, 4 cationic, 6 nonionic) were examined for potential eye mucosa irritation by the method of Draize, Woodard and Calvery (*J. Pharmacol. & Exper. Therap.* 82: 377, 1944). Variations in toxicity were noted among the 3 broad groups of surface-active agents, and among members of the same general groups. In general, the toxicity of the groups was in the following decreasing order: cationic > anionic > nonionic. The inclusion of several surface-active agents in a formulation, especially if these represented members with different ion activity, may produce a preparation of greater or less toxic properties than would be anticipated from the simple addition of toxic effects of the component surface-active agents.

Convulsant action of diols related to diethyl propanediol. PETER E. DRESEL,* IRWIN H. SLATER, E. J. PRIBYL* AND J. F. O'LEARY.* *Divs. of Orthopedic and Pharmacology, Univ. of Rochester School of Medicine, and the Research and Development Labs., E. R. Squibb and Sons, Rochester, N. Y.*

2,2-diethyl 1,3-propanediol is a central nervous system depressant acting selectively on multisynaptic pathways. It is particularly effective against convulsions caused by pentylenetetrazole. Among a series of diols of longer chain length we have found 2,2-diethyl 1,4-butanediol (MC 2973), 2,2-diethyl 1,5-pentanediol, and 3,3-diethyl 1,5-pentanediol (MC 3206) to be of special interest. MC 2973 was found to be a potent central nervous system stimulant which in mice caused clonic and tonic convulsions only slightly different from those caused by pentylenetetrazole. The median lethal dose on intraperitoneal administration was 32 mg/kg. In very large doses, such as 1400 mg/kg. the convulsant activity was replaced by a rapidly fatal depression. MC 3206 shows activity similar to MC 2973, but was not as potent. MC 3175, on the other hand, causes a period of depression followed by clonic-tonic convulsions. Convulsions and death by these agents can be protected against by diethyl propanediol. In the case of MC 3175, the antidote was administered in the interim between depression and convulsion. This protection is not specific, since phenobarbital also protects these animals. The fact that one of these simple compounds shows depressant activity, another stimulant activity and the third mixed activity suggests that these diols may prove a very useful tool in the study of drug action in the central nervous system.

Preliminary biochemical studies will be presented. It appears that oxygen uptake of rat brain homogenates with or without added pyruvate is inhibited by rather high concentrations of diethyl propanediol.

Influence of nitrogen mustards on citric acid synthesis. KENNETH P. DUBOIS AND KENNETH W. COCHRAN.* *U. S. Air Force Radiation Lab. and the Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.*

Recent studies in this laboratory (*Proc. Soc. Exper. Biol. & Med.* 76: 422, 1951) demonstrated that x-irradiation of male rats causes a marked decrease in the ability of hematopoietic tissues to synthesize citric acid and an increase in citrate formation in liver as measured by the fluoroacetate technique of Potter (*Proc. Soc. Exper. Biol. & Med.* 76: 41, 1951). The radiomimetic properties of nitrogen mustards suggested that similar changes might follow poisoning by these toxicants. For this investigation groups of rats were given various doses of ethyl-*bis*(2-chlorethyl) amine hydrochloride intraperitoneally. At intervals during the first 5 days following treatment with the nitrogen mustard groups each containing 6 animals were given fluoroacetate and sacrificed 3 hours later for citric acid measurements. In 24 hours after 5 mg/kg. of the nitrogen mustard the synthesis of citrate was decreased 72% and 74% in spleen and thymus respectively while liver showed a marked increase. With decreasing doses of nitrogen mustard similar but less prominent effects were obtained in spleen and thymus as evidenced by 18% and 26% inhibition of citrate accumulation at 3 days after 0.75 mg/kg. of the nitrogen mustard. The increase in the value for liver remained prominent after this dose of the toxicant. Toxic doses of methyl-*bis*(2-chlorethyl) amine produced similar effects. The results of these experiments demonstrated a similarity between x-irradiation and nitrogen mustards in their ability to alter citrate synthesis in rat tissues *in vivo*.

Inhibitory action of Systox on cholinesterase. KENNETH P. DUBOIS, JERE A. DEROIN* AND ARTHUR J. OKINAKA.* *Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.*

Systox (1059) is a new organic phosphorus-containing insecticide which exhibits both contact and systemic insecticidal activity as well as a fumigant action. It is the diethoxy thiophosphoric ester of 2-ethyl mercapto ethanol and is thus chemically related to Parathion. The present investigation was undertaken to obtain information on the pharmacological actions of Systox in mammals. For *in vitro* studies solutions of Systox (Geary Chemical Corp.) in 10% ethanol and 90% propylene glycol were employed. The approximate LD_{50} of this compound given intraperitoneally to female rats was 3 mg/kg. Acutely toxic doses of Systox produced a rapid onset of generalized cholinergic effects resembling the actions of Parathion. A strong inhibitory effect on cholinesterase was demonstrated by *in vitro* tests in which 50% inhibition of rat brain cholinesterase was obtained with a final concentration of 5×10^{-7} M Systox. At 1 hour after intraperitoneal administration of 1 mg/kg. of Systox to female rats the cholinesterase activity of brain and submaxillary later the inhibition had decreased to 35% in brain and 39% in submaxillary gland indicating reverxibility of the inhibition *in vivo*. At the time of death in approximately 45 minutes after intraperitoneal administration of 5 mg/kg. of Systox the cholinesterase activity of brain and submaxillary gland was 85% inhibited and the enzyme activity of serum was also markedly inhibited. These studies indicated that Systox is a potent inhibitor of cholinesterase *in vitro* and *in vivo* producing both nicotinic and muscarinic effects in mammals.

Some blood component relationships in mental patients following exercise. O. D. EASTERDAY,* R. M. FEATHERSTONE, J. S. GOTTLIEB* AND R. V. HOGG.* *Depts. of Pharmacology, Psychiatry, and Mathematics, State Univ. of Iowa, Iowa City.*

It has been noted that patients with schizophrenia fail to respond to sodium amytal clinically as do patients with manic-depressive psychosis, depressive type. Preparatory to using sodium amytal as a stress to be placed on patients with mental diseases during a study of their oxidative mechanisms, the previous suggestion that exercise affected lactic acid-glutathione relationships in patients with schizophrenia differently than in normal controls was reviewed experimentally in this laboratory but not confirmed. A statistical evaluation and comparison of the changes that occurred in the blood values of reduced glutathione, lactic acid, and pyruvic acid after ergometer-measured exercise among 10 normal control subjects, 10 patients with schizophrenia, and 10 'other' patients with either manic-depressive psychosis-depressive type, or psychoneurosis will be presented. Lactic and pyruvic acid values following exercise were significantly lower in normal subjects than in patients with schizophrenia. Several factors not easily controlled were evaluated through determination of correlation coefficients for all possible combinations among time periods, height, weight, age, surface area, exact amount of work done, lactic acid, pyruvic acid and reduced glutathione. The bearing of a high incidence of significant correlations among these variables for the group of patients with schizophrenia in contrast to a corresponding

low incidence in the group of normal subjects and an intermediate incidence for the 'other' group on the possible functioning of more uniform biological mechanisms in schizophrenia will be discussed.

A morpholinium analogue of C_{10} and its antagonism by neostigmine. OTHO D. EASTERDAY,* CALVIN HANNA* AND F. W. SCHUELER. *Dept. of Pharmacology, State Univ. of Iowa, Iowa City.*

In order to investigate the possible role of negative polarizing groups adjacent to the quaternary nitrogen in the neostigmine antagonism of D-tubocurarine compared to C_{10}, decamethylene bis (n-methyl morpholinium) dibromide (MC_{10}) has been synthesized. This material possesses negative polarizing ether-oxygen functions two methylene groups away and adjacent to quaternary nitrogen atoms. Curariform respiratory depression by this material is antagonized by neostigmine in the dog and the head drop dose of MC_{10} in rabbits is more than doubled by the previous administration of 30 micrograms/kg. of neostigmine. The relationships are well demonstrated by the following data in which MC_{10}, C_{10}, and d-tubocurarine are compared. H.D.D., *d*-tubocurarine, 0.18 mg/kg.; C_{10}, 0.14 mg/kg.; MC_{10}, 0.88 mg/kg. H.D.D. following 30 μg/kg. of neostigmine; *d*-tubocurarine, 0.43 mg/kg.; C_{10}, 0.17 mg/kg.; MC_{10}, 1.96 mg/kg. The LD_{50} of MC_{10} in mice by the intraperitoneal route was 2.3 mg/kg. MC_{10} produced in pigeons the paralysis typical of *d*-tubocurarine in contrast to the rigidity characteristic of C_{10}.

Local anesthetic activity of some substituted glycinamides. DONALD K. ECKFELD,* ALBERT J. BEGANY,* JEROME M. GLASSMAN* AND JOSEPH SEIFTER. *Wyeth Inst. of Applied Biochemistry, Wyeth Inc., Philadelphia, Pa.*

Approximately 300 N,N′ substituted glycinamides have been prepared and screened for various pharmacological properties. Of these, 100 were from one-tenth to several times as potent as cocaine in producing local anesthesia by topical application to the rabbit cornea when applied as a 0.25–1.0% solution. The presence of an aromatic substituent was not essential for local anesthesia. Substitution of either an aliphatic or pressor amine residue usually enhanced the local anesthetic action, particularly with branching of the alkyl portion of the sympathomimetic amine. In a few instances the glycinamides derived from sympathomimetic amines had both pressor and local anesthetic action. Dibenamine reversed the pressor activity of some of these without affecting the local anesthesia. The toxicity of the glycinamides selected for clinical trial was one half to one third that of cocaine by intraabdominal injection in albino mice. Other pharmacological actions of these compounds will be discussed.

Pharmacologic responses of the isolated rabbit heart during x-irradiation. L. E. ELLINWOOD,* J. E. WILSON* AND J. M. COON. *U. S. Air Force Radiation Lab. and Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.*

Atypical responses of the isolated rabbit heart to various drugs were observed after the heart had been x-irradiated (250 kv) with 34,000 r or more. Injections of epinephrine-HCl produced a diphasic reaction characterized by a cardiac arrest following the acceleration. A diphasic response was also regularly produced by the injection of acetylcholine after atropinization. This response differed from the normal effect of ACh in the atropinized heart in that a marked acceleration was followed by a temporary arrest in diastole. The administration of nicotine produced a triphasic response (arrest-stimulation-arrest) instead of the normally occurring diphasic one (arrest-stimulation). The first of these phases was eliminated by previous atropinization of the heart. The responses to epinephrine, and to ACh and nicotine following atropinization, were indistinguishable. A loss of potassium from the heart during irradiation was also observed. The rate of potassium release in the perfusate reached a peak after a dose of about 2000 r delivered at 500 r/minute. It then gradually decreased to near the control level and fluctuated slightly throughout the period of experimentation. The greatest average increase of potassium in the perfusate was 11% over the average control. At this rate the irradiated isolated heart loses approximately 25% of its potassium in an hour following x-ray exposure. The possibility that the characteristic arrest following the acceleration induced by the drugs tested in the irradiated isolated heart is due to loss of potassium is being explored.

Observations on bronchodilator activity of visammin (khellin). FRED W. ELLIS. *Dept. of Pharmacology, Univ. of North Carolina School of Medicine, Chapel Hill.*

Changes in bronchial tone of intact dog lungs were recorded by the use of Jackson's lung plethysmograph and negative artificial respiration. The animals were anesthetized with pentobarbital sodium, both vagus and phrenic nerves were sectioned and small doses of dihydro-beta-erythroidine were injected to suppress intercostal and accessory respiratory movements. Bronchoconstriction was induced by intravenous injections of methacholine-physostigmine or histamine solutions. Visammin was relatively ineffective in antagonizing or preventing cholinergic constriction. Its prophylactic potency against challenging doses of histamine was greater and lasted longer than the protection afforded by aminophylline in comparable doses. Under similar conditions the

protective effect of visammin was less potent but of longer duration than that of isopropylarterenol.

Autonomic drugs and liver slices. SYDNEY ELLIS AND HILTON L. ANDERSON.* *Dept. of Pharmacology, Temple Univ. School of Medicine, Philadelphia, Pa.*

The studies of Sutherland and Cori (*J. Biol. Chem.* 188: 531, 1951) concerning the effect of epinephrine and a few other sympathomimetics on the production of glucose by rabbit liver slices have been extended to include the effects of a larger number of sympathomimetics on liver slices obtained from rabbits and rats. Results were similar for both species. Minor differences will be correlated with the *in vivo* activities of the sympathomimetics. In general, catechol and phenol containing sympathomimetics were active, and phenyl and aliphatic sympathomimetics, as well as catechol itself, were inactive. Several adrenergic-blocking drugs were tested on rabbit liver slices. Ergotamine, dihydroergotamine and ergotoxine antagonized the action of epinephrine; whereas Dibenamine, SY-28, Priscoline and Regitine did not antagonize the action of epinephrine. Ephedrine potentiated the action of epinephrine. Carbachol did not affect glucose production. Atropine had no direct action on the liver nor did high concentrations interfere with the action of epinephrine.

Anticonvulsant action of CO_2 on various types of seizures. G. M. EVERETT, J. S. GOODSELL* AND J. E. P. TOMAN. *Dept. of Pharmacology, Abbott Labs., North Chicago, Ill.*

In view of the reported usefulness of CO_2 in status epilepticus and against petit mal, observations have been made in mice subjected to supramaximal electroshock (ES), psychomotor shock (PS) and Metrazol (M) seizures, after 10-minute exposures in a chamber with controlled CO_2/air mixtures. Concentrations up to 10% CO_2, producing hyperpnea, are without effect on seizures. Fourteen per cent CO_2, producing slight ataxia, eliminates the tonic extensor phase of ES and M. However, the tonic flexor and clonic components are still present. The PS are not suppressed at this level, but are eliminated at 20% CO_2. Above 22% CO_2, which causes ataxia and decreased activity, all tonic components of ES and M are abolished, but clonic convulsions persist up to levels of CO_2 producing coma. The most pertinent feature in the relatively weak anticonvulsant action of CO_2 seems to be increase in threshold. In rabbits (epidural electrodes) the threshold for focal seizures is increased by inhalation of CO_2 sufficient to produce hyperpnea and neurological depression. In frog sciatic nerve, threshold is increased and conduction velocity slowed with CO_2 exposure insufficient to change other properties relevant to seizure phenomena, such as rebound spikes and facilitation.

Circulatory effects of N-ethyl-3-piperidyl-diphenylacetate hydrochloride. PAUL L. EWING AND LLOYD D. SEAGER. *Dept. of Pharmacology, College of Pharmacy, and Dept. of Physiology and Pharmacology, Univ. of Arkansas School of Medicine, Little Rock.*

Intravenous doses of 2–4 mg/kg. of N-ethyl-3-piperidyl-diphenyl acetate hydrochloride (JB-305, Lakeside) in dogs produce only moderate changes in blood pressure. Usually there is a transient fall followed by a moderate rise. As the dose is increased above this level, the depressor phase becomes accentuated. Doses of 8 mg/kg. produce a marked but temporary fall in pressure while doses of 12–16 mg/kg. cause a profound and more persistent fall in pressure accompanied by cessation of respiration. Salivary secretory response to chorda tympani stimulation and cardiac slowing to vagal stimulation are diminished or abolished after 4 mg/kg. of the drug. Depressor responses to acetylcholine are not diminished even after 12 mg/kg. of the drug. The pressor response of nicotine is lessened or abolished by doses of 6 or more mg/kg. Metahydroxypropadrine and other epinephrine-like compounds antagonize the depressor effect of JB-305. In turtles and frogs, JB-305 abolishes the effect of vagal stimulation but does not modify the effect of white-line stimulation in the frog. Intravenous administration in turtles also produces slowing of the cardiac rate, A–V block and weakening of both auricular and ventricular contractions.

Some effects of mercurial diuretics on renal sodium excretion. ALFRED FARAH AND FLORENCE KODA.* *Dept. of Pharmacology, New York State College of Medicine, Syracuse, N. Y.*

The increase in sodium excretion produced by mercurial diuretics is usually produced by an increase in urinary sodium concentration and by an increase in urine volume. There is a correlation between maximum urinary sodium concentration after the mercurial mersalyl and the plasma sodium concentration. Acute reduction of plasma sodium concentrations to levels of about 80 mM/l. resulted in a refractoriness of the animal to the mercurial. Under these conditions sodium cyanide in a dose of 0.5 mg/kg. still produced a marked increase in urinary sodium concentration and excretion. It is concluded that at least two sodium reabsorptive mechanisms exist in the kidney; *a*) a mercury sensitive one which is abolished by reduced sodium filtration or reduced plasma sodium concentration but is intensified by pretreatment with ACTH or DOCA or sodium chloride infusions; *b*) a mercury resistant but cyanide sensitive sodium reabsorption mechanism. One to two

milligrams per kg. of mersalyl induce a natriuresis with no change or a slight reduction in water excretion. BAL in carefully graded small doses reverses the urine volume changes produced by large doses of the mercurial but will not reverse the urinary sodium concentration changes thus produced. This indicates that mercurial diuretics have at least two sites of action in the renal tubules.

Electroencephalographic patterns and arterial ether concentrations: A correlation during anesthesia. ALBERT FAULCONER, JR. *Section of Anesthesiology and Intravenous Therapy, Mayo Clinic, Rochester, Minn.*

The presentation is of a correlation between the Courtin, Bickford, Faulconer classification of electroencephalographic patterns and concentrations of arterial blood ether simultaneously observed during anesthesia of increasing depth effected with ether-oxygen or with ether-oxygen-nitrous oxide. Results of this study can be summarized as follows: *1*) Almost a straight line correlation was revealed in 225 observations on 31 patients undergoing ether anesthesia, with or without nitrous oxide, when the mean of the blood ether concentrations observed at each electroencephalographic level were plotted against the electroencephalographic levels. The points on this correlation graph ranged from a mean arterial blood ether concentration of 52 mg/100 cc. at electroencephalographic level 1 to a mean of 120 mg/100 cc. at electroencephalographic level 5. *2*) A lower ether concentration was required to produce each of the electroencephalographic levels studied when nitrous oxide was present in the arterial blood in excess of 10 mg/100 cc. than when no nitrous oxide was present. This difference was shown to be statistically significant to a high degree for the observations made at electroencephalographic levels 4 and 5. It is suggested that this method of analysis is adaptable to the quantitative study of the contribution of each of two narcotic agents when used simultaneously, provided a significant electroencephalographic pattern change subject to classification is produced by the agents considered. *3*) No significant difference could be determined between the means of arterial blood ether concentrations required to produce specific electroencephalographic levels, in the absence of nitrous oxide, whether the observations were made earlier than 45 minutes after the beginning of anesthesia or later than 45 minutes after the beginning of anesthesia.

Effect of colchicine on frog skeletal muscle. FRANK C. FERGUSON, JR. *Dept. of Pharmacology, Cornell Univ. Med. College, New York City.*

Colchicine alters the metabolism of isolated frog skeletal muscle. Oxygen consumption of resting sartorius muscle is approximately doubled by colchicine (1.6×10^{-2} M). After activation by caffeine or dinitrophenol, oxygen consumption is increased by a previously ineffective level of colchicine (4×10^{-3} M). Higher levels of colchicine in caffeinated muscle produce no greater effect and the increase is not well sustained. When the caffeine concentration is increased to a level which consistently causes contracture, colchicine produces no further increase in oxygen uptake. Sodium azide blocks the stimulating effects of both caffeine and colchicine. Anaerobic glycolysis in caffeinated muscle is also stimulated by colchicine in the same dose range. Contractile force of oxygenated, curarized sartorius muscle, stimulated directly, is unaffected by colchine in 10^{-3} M concentration but a concentration of 4×10^{-3} M produces a sustained increased in the contraction. This increase in force does not occur in the absence of a liberal supply of oxygen. With a colchicine concentration of 1.6×10^{-2} M, failure of contraction begins within 2 hours and muscles are unable to maintain tetanic contractures for more than a few seconds. Further studies are in progress.

Interaction of hyaluronidase with thromboplastic components of blood coagulation. SILVIO FIALA AND KARL ROTH (introduced by F. M. BERGER). *Dept. of Research, Albert Einstein Med. Center, Philadelphia, Pa.*

Dialyzed bovine hyaluronidase when incubated with human plasma prolongates or inhibits its clotting. Enzyme heated to 60°C. for 15′ has no activity. Prothrombin time is not influenced by the enzyme which indicates that prothrombin, labile factor and fibrinogen are not attacked by hyaluronidase. The inactivating effect of the enzyme is counteracted by tissue (brain) thromboplastin and also by the addition of washed isolated platelets. On the other hand incubation of platelets with hyaluronidase leads to their loss of thromboplastic activity. Thromboplastic components of both plasma and of platelets bind the enzyme competitively with resulting inhibition of the clotting mechanism and hyaluronidase. The platelets in addition to protein material inactive in coagulation contain an active thermostable material which has an affinity for hyaluronidase. Tissue thromboplastin interacts with hyaluronidase also. The significance of these findings for the importance of the carbohydrate component of thromboplastic activity will be discussed.

Effect of salicylates on experimental beryllium poisoning. ASHER J. FINKEL AND MARCIA R. WHITE (introduced by NICHOLAS W. FUGO). *Argonne Natl. Lab., Chicago, Ill.*

Acute experimental beryllium poisoning in mice, readily produced by the intravenous injection of soluble beryllium salts, usually terminates fatally

in 3 days. In repeated series of experiments, intravenously administered sodium salicylate (400–600 mg/kg.) gave 70–100% protection against LD_{95} amounts of intravenous $BeSO_4$ (0.7 mg/kg. of Be) when injected 1 hour after the toxic agent. Sodium salicylate doses of 200 mg/kg. were approximately 60% effective. Protection was afforded with 400 mg/kg. when sodium salicylate treatment was delayed 8 hours but not if treatment was postponed 24 hours. Pretreatment 8 hours before beryllium was administered was ineffective. Preliminary experiments with sodium para-aminosalicylic acid (400 mg/kg.) given 1 hour after the beryllium produced equivocal results. Protection is believed to result from the chelating action of the ortho-hydroxy-carboxylic acid structure, which reduces the availability of the toxic ionized beryllium by the formation of un-ionized complexes.

Toxicological observations on two quaternary ammonium germicides. J. K. Finnegan, P. S. Larson, R. Blackwell Smith, Jr., H. B. Haag, J. Douglas Reid* and M. L. Dreyfuss*. *Depts. of Pharmacology and Bacteriology, Med. College of Virginia, Richmond; and Pathology Lab., Chesapeake and Ohio Hosp., Clifton Forge, Va.*

Two quaternary compounds, di-isobutyl-phenoxy-ethoxy-ethyl dimethylbenzyl ammonium chloride (Hyamine 1622®), and methyl-dodecylbenzyl trimethyl ammonium chloride (Hyamine 2389 ®), have been studied as to their acute and chronic toxicities, their effect on intestinal flora and water retention, and on intestinal tone and motility. In rats the acute oral LD_{50} of Hyamine 1622 is 420 ± 25 mg/kg., and for Hyamine 2389, 325 ± 65 mg/kg. Intraperitoneally the LD_{50} of the latter is 7.9 ± 0.4 mg/kg. and intravenously 1.9 ± 0.1 mg/kg. One-year feeding studies were done in dogs at dietary concentrations of 5, 100 and 500 ppm using 3 animals at each level. All animals gained weight and blood studies revealed no abnormalities at the end of the feeding period. Histopathologic studies are in progress. Two-year feeding studies in rats are under way using dietary concentrations of 0, 50, 200, 1000, 2500, and 5000 ppm. Hematologic studies made at 11 months gave values within normal limits. At 15 months mortality was significantly higher only at the 5000 ppm. levels. Some growth depression occurred at 2500 ppm. Animals dying revealed ceca distended with gas and very fluid intestinal contents. On the isolated intestine Hyamine 1622 depressed reversibly both tone and contractility. Gram-positive cecal and fecal flora decreased at levels of both compounds of 2500 and 5000 ppm. A relative increase in *E. coli* and/or *A. aerogenes* and *P. vulgaris* flora occurred. The relative number of aerobic and anaerobic and the total number of bacteria was unchanged.

Estrogenic action of DDT and some analogues. Allan L. Fisher,* Hugh H. Keasling* and F. W. Schueler. *Dept. of Pharmacology, State Univ. of Iowa College of Medicine, Iowa City.*

Burlington and Lindeman (*Proc. Soc. Exper. Biol. & Med.* 74: 48, 1950) reported that DDT retarded growth of comb, wattle and testes in the White Leghorn Cockerel. They speculated that DDT may have an estrogenic action. This possibility was of interest in the light of the working hypothesis by Schueler (*Science* 103: 221, 1946) pertaining to the relationship between chemical constitution and estrogenic action. Since the structure of DDT does not fulfill the requirements proposed by this hypothesis for estrogenic action, we have tested DDT and its analogues, methoxychlor and 2,2′ bis (p-hydroxy phenyl) 1,1,1, trichloroethane for estrogenic action in ovarectomized rats. The drugs were given subcutaneously in 3 divided doses. Total doses were 15, 30 and 45 mg. for DDT and methoxychlor and 10, 15, 20, 25 and 45 mg. for the dihydroxy analogue. DDT and methoxychlor were inactive at the above doses. The dihydroxy analogue elicited an estrus response at all tested doses. From these data it appears that DDT does not possess direct estrogenic action. The dihydroxy analogue while somewhat short (12.5 Å) for maximal estrogenic action would be expected to show some activity according to the working hypothesis. Actually it is of high potency relative to non-halogen diphenyl methane derivatives. This may be due to an inductive and/or field effect of the chlorine atoms acting on the phenolic hydroxyl group. Experiments designed to test this possibility are in progress. (This work was aided by a grant from the United States Public Health Service.)

Some observations on the chronic toxicity of Antabuse (tetraethylthiuramdisulfide). O. G. Fitzhugh, W. J. Winter* and A. A. Nelson.* *Div. of Pharmacology, Food and Drug Admin. Federal Security Agency, and Veterans Admin. Washington, D. C.*

In experiments lasting from 8 weeks to 2 years albino rats were given dosage of 100, 300, 1000 and 2500 ppm Antabuse in their diet. Gross symptoms of ataxia occurred in some rats on the 2500 ppm dosage as early as 8 weeks. These symptoms in some instances progressed to partial paralysis and finally death. All animals on the two higher dosages and a few of those on the other dosages showed symptoms of muscular incoordination within the 2-year period. Histopathological examination demonstrated numerous calcified masses up to 0.3 mm. in diameter in the granular layer of the cerebellum, basal ganglia, and, to a

lesser extent, elsewhere in the brains of rats on 2500 ppm Antabuse. Preliminary examination of the viscera and other tissues including skeletal muscle has not shown any marked histopathological damage. At 2500 ppm Antabuse also produced marked effects on growth and mortality rates. Lower dosages showed a slight to moderate effect on growth.

Acute cardiovascular refractoriness induced by epinephrine. PATRICIA FLAUM & WILLIAM G. CLARK. *Dept. of Physiological Chemistry, Univ. of California School of Medicine, and Veterans Admin. Center, Los Angeles.*

A marked refractoriness to cardiovascular responses to epinephrine and related drugs has been induced in animals under Dial or pentobarbital anesthesia by doses of 1–100 mg/kg. of epinephrine and related drugs given by intra-arterial or intravenous infusion or by stepwise acute injections. A total of over 250 animals has been studied in experiments of 5–10 hours' duration. The majority were cats, but dogs, rabbits and rats were included. This refractoriness is not mediated by: *1*) paralysis of the autonomic nervous system through prolonged pressor insult, from irreversible shock, or from damage to the central nervous system—however, the peripheral receptors are involved since adrenergic blocking drugs prevent its induction; *2*) production of known depressor substances such as ferritin, histamine or anoxic metabolites; *3*) accelerated inactivation of epinephrine *in vivo*. Consequent to these findings evidence was obtained that the mediator may be a blood-borne factor, present in the non-protein serum fraction. Factors influencing the production and duration of refractoriness are: *1*) sex; refractoriness is more easily induced in the male than in the female, especially the pregnant female—this seems correlated with the more rapid fall in blood epinephrine in the male and the indicated ability of the female to show elevated blood sugar levels and to maintain them longer; *2*) total amount and rate of administration of inducing drug—the higher the dose, the longer the duration; infusion is more effective than the equivalent dose given acutely; *3*) administration of i.v. dextrose; dextrose infusion prolongs refractoriness and reestablishes it after it disappears; *4*) molecular configuration of inducing drug; the 3 or 4 phenolic hydroxyl, the N-methyl and the beta hydroxyl groups are important; *5*) presence of hypertension—induction seems more difficult, and duration of refractoriness shorter in stock animals with initial blood pressures exceeding 180–200/100–200. (Supported by grants from USPHS and Life Insurance Medical Research Fund.)

Renal hemodynamics as effected by posture and by sympathoadrenal blockade. RALPH V. FORD* AND JOHN H. MOYER. *Depts. of Medicine and Pharmacology, Baylor Univ. College of Medicine and the Veterans Admin. Hospital, Houston, Texas.*

The renal effects of orally administered N-phenoxyisopropyl -N- benzyl -β- chlorethylamine hydrochloride were evaluated both in the supine and tilted (60°) positions on 6 patients with hypertension. Renal plasma flow (PAH clearance), glomerular filtration rate (inulin clearance), and maximum tubular function (TmPAH) were studied. In none of the patients was the blood pressure depressed significantly by the drug in the supine position. However, 5 of these showed a significant acute orthostatic hypotensive response which was interpreted as indicating adrenergic blockade. The control renal studies revealed impairment of renal function as reflected in abnormally low GFR, RPF, and TmPAH. After the drug, one significant effect on renal function in the supine position was an 18% increase in GFR, the significance of which was not apparent. During control tilt studies the mean GFR and RPF decreased proportionally (13% and 18% respectively) without a depression in blood pressure. After the drug in the tilted position, there was a greater decrease in GFR (28%) and RPF (25%), which was proportional to the drop in blood pressure (25%). Changes in TmPAH were not significant. It was concluded that the further depression in renal function in the tilted position after the drug was purely due to depression of blood pressure. Although there appeared to be sympathetic blockade as indicated by the orthostatic hypotension, there was no selective renal neurogenic blockade either in the supine or in the tilted position.

N-allylnormorphine in treatment of methadone poisoning in man: report of two cases. H. F. FRASER,* ABRAHAM WIKLER, A. J. EISENMAN AND HARRIS ISBELL. *NIMH Addiction Research Center, USPHS Hosp., Lexington, Ky.*

Case 1. A Negro male aged 33, a former heroin addict, volunteered for an experiment in which he received 20 mg. methadone intravenously at 8:30 A.M. and again at 1:30 P.M. The latter injection induced nausea and weakness followed by sedation which progressed to deep sleep at 4:30 P.M. At 6 P.M. patient was comatose and respiratory rate was 3/minute. Neither artificial respiration nor 375 mg. nikethamide intramuscularly benefited the patient. At 7 P.M. deep tendon, gag and corneal reflexes could not be elicited and respiratory rate was 2/minute. Rectal temperature was 35.8°C, pulse was of good quality and the rate was 75/minute. Patient was given 40 mg. N-allylnormorphine intravenously. Four minutes later respiratory rate rose to 10/minute and 23 minutes after injection, rate was 23/minute. Seventy minutes after

administration of N-allylnormorphine patient was very drowsy but could be aroused by vigorous stimulation. Shortly afterward he walked with assistance Recovery was progressive and uneventful. Concurrently, another healthy Negro male received from the same bottle 20 mg. methadone intravenously at 8:30 A.M. and again at 1:30 P.M. He showed no unusual response. *Case* 2 is analogous to *case* 1 and will be described. These two cases illustrate the cumulative effects of successive, relatively large doses of methadone and the reversal of the resulting respiratory depression and coma by N-allylnormorphine. Since this amount of methadone has been given to more than 125 other post-addicts without significant poisoning, a considerable variation of susceptibility occurs.

Effect of liver damage on toxicity of octamethylpyrophosphoramide (OMPA). JOHN P. FRAWLEY,* EDWIN P. LAUG AND O. GARTH FITZHUGH. *Div. of Pharmacology, Food and Drug Admin. Federal Security Agency, Washington, D. C.*

It has been believed that the major toxic effects of OMPA in mammals is due to liver conversion of OMPA to a cholinesterase inhibitor. *In vitro* studies demonstrate a lack of inhibitory activity, while, *in vivo*, marked cholinesterase inhibition occurs. DuBois *et al.* (*J. Pharmacol. & Exper. Therap.* 99: 3, 1950) have shown that partially hepatectomized rats are somewhat more resistant to OMPA than intact animals. We have further demonstrated by *in vivo* studies that this increased resistance to OMPA can be produced by the administration of carbon tetrachloride and that the increased resistance is directly related to a decreased cholinesterase inhibition. Eight female rats were administered 1 ml/kg. orally of carbon tetrachloride 20 hours prior to an LD_{100} dose (33 mg/kg.) of OMPA. A similar number of control females received only an LD_{100} dose of OMPA. Within 2 hours all controls had died whereas even the typical parasympathomimetic symptoms were absent in the carbon tetrachloride poisoned animals. Between 2 and 6 hours symptoms developed in these animals and 4 succumbed within 24 hours. When carbon tetrachloride poisoned male rats were administered 2 mg/kg. of OMPA orally, only 32% inhibition of erythrocyte cholinesterase occurred after 4 hours, whereas the same dose of OMPA caused 63% inhibition in normal animals. Similar effects have been observed with either sex, and the degree of protection appears to be directly related to the dose of carbon tetrachloride and the degree of liver damage.

Mechanism of toxicity of thioglycolate. MARIAN V. FREEMAN AND ROBERT M. ROSENTHAL (introduced by J. H. DRAIZE). *Div. of Pharmacology, Food and Drug Admin. Federal Security Agency, Washington, D. C.*

Because thioglycolate is being used extensively in the permanent waving of hair and has been suggested for the treatment of radiation sickness, the mechanism of its toxicity is being investigated. Grossly, animals administered thioglycolate exhibit depression and in fatal cases, convulsions with respiratory failure. Blood sugar was determined on rabbits, rats and dogs. In all 3 species there was a severe fall in blood sugar. Sugar analysis of the urine established that the lowering of blood sugar was not the result of a decrease in renal threshold for glucose. The intraperitoneal LD_{50} of sodium thioglycolate was determined on rats with and without dextrose therapy. One cc. of a 25% solution of dextrose was injected at the time of the sodium thioglycolate injection and at 2 hourly intervals for 6 hours thereafter. The LD_{50} of sodium thioglycolate with dextrose therapy was found to be 390 mg/kg. as opposed to 148 mg/kg. without dextrose.

Influence of diethylstilbestrol and spaying on antidiuresis following vasopressin. ROBERT W. GARDIER* AND ROBERT A. WOODBURY. *Div. of Pharmacology, Univ. of Tennessee, Memphis.*

Species differences were observed between the dog and rat. In the hydrated female dog diethylstilbestrol (5 mg/kg. i. m. twice daily for 3–6 days) prolonged and increased by 50% the intensity of the vasopressin antidiuresis (66 milliunits/kg. subcutaneously). This occurred without significant change in the PAH clearance and in spite of a 40% increase in the creatinine clearance. In the absence of vasopressin, diethylstilbestrol increased the urine flow 150–350% above control levels. Creatinine clearance in these studies increased 35–45% and PAH clearance increased 15–25% above the control levels. Changes in glomerular filtration and effective plasma flow cannot account for all of the increased urine flow, or for the increased vasopressin antidiuresis. In hydrated female rats diethylstilbestrol (5 mg/kg. i. m. twice daily for 3 days) produced no significant effect on the vasopressin antidiuresis (66 milliunits/kg. subcutaneously). Similar test doses of vasopressin to hydrated spayed rats, however, did produce in 105 minutes a 51% greater antidiuretic effect which disappeared by the 135-minute period. After treatment of these spayed rats with diethylstilbestrol, the antidiuretic response to vasopressin was potentiated at not only the 105-minute period but to the end of the observation period at 165 minutes. Without vasopressin diethylstilbestrol did not increase the urine flow in either the spayed or non-spayed rats. In rats caffeine citrate in oral doses of 10 mg/kg. shortened the antidiuresis after vasopressin only when animals were spayed and had received no diethylstilbestrol.

Action of enzyme inhibitors on sinus rate, contractility, electrical excitability, refractory period and conduction velocity in isolated rabbit auricle. ELMER A. GARDNER* AND ALFRED FARAH. *Dept. of Pharmacology, New York State College of Medicine, Syracuse, N. Y.*

Isolated rabbit auricles were suspended in Locke's solution at 30°C. The above properties of the muscle were recorded by standard methods on an inkwriting oscillograph. Anoxia produced a reduction in all the above properties but increased the effective refractory period. Contractility is effected more than the other properties of heart muscle studied. The increase in the effective refractory period during anoxia was frequently preceded by a decrease. Following recovery from anoxia the effective refractory period was frequently decreased below control levels. The rate of change in the above functions following anoxia depended on the amount of buffer in the Locke solution. Cyanide acts similar to anoxia affecting contractility earlier and to a greater extent than the other cardiac properties studied. NaFAc and dinitrophenol produce effects qualitatively similar to cyanide. Iodoacetate in a concentration of 0.0004 M produced a marked reduction in sinus rate and lesser effects on contractility. The chronotropic effect of epinephrine was markedly reduced while the inotropic effect was not affected by pretreatment with iodoacetate. The addition of lactate pyruvate or acetate partially overcomes the effects of iodoacetate on sinus rate while having minimal effects on the height of contraction.

Effects of thyroxine on oxidation of ascorbate and succinate by cytochrome oxidase system. CHALMERS L. GEMMILL. *Dept. of Pharmacology, Univ. of Virginia Med. School, Charlottesville.*

Using a freshly prepared, centrifuged and diluted homogenate from rat's heart, it has been shown that the oxidation of ascorbate by the cytochrome oxidase system is inhibited by thyroxine. The degree of inhibition is inversely proportional to the concentration of cytochrome *c*. With succinate as a substrate, thyroxine stimulates the uptake of oxygen in the system rat heart homogenate—cytochrome *c*. However, thyroxine inhibits the succinate dehydrogenase system when methylene blue is used as an electron acceptor. When ascorbate is added to the succinate-heart homogenate-cytochrome *c* system an increment in oxygen consumption is obtained over the control without ascorbate. An additional increment is obtained when both thyroxine and ascorbate are added to this system. The final concentration of thyroxine used in these experiments was 50 micromols. In order to explain these results, it is assumed that thyroxine forms a reversible oxidation-reduction system with its potential between the ascorbate and cytochrome *c* systems under the conditions of these experiments.

Effect of hyaluronidase on inflammatory response to primary irritants. JEROME M. GLASSMAN,* ALBERT BLUMENTHAL,* WILLIAM J. BECKFIELD* AND JOSEPH SEIFTER. *Wyeth Inst. of Applied Biochemistry, Wyeth Inc., and the Graduate School of Medicine, Univ. of Pennsylvania, Philadelphia, Pa.*

Paraphenylenediamine hydrochloride dissolved in physiological salt solution, and allylisothiocyanate dissolved in mineral oil were injected with and without hyaluronidase intradermally in 22 rabbits. Trypan blue was then injected intravenously to observe the effect on vascular permeability in the treated areas. Hyaluronidase caused a spread of both the aqueous and oil-soluble irritants as indicated by the greater area in which dye accumulated. With the aqueous irritant (paraphenylenediamine) hyaluronidase prevented the gross edema and decreased the fibrinous exudate and destruction of underlying tissue. Tissue repair was more rapid. These benefits were probably due to reduction of effective concentration by spreading action and more rapid absorption of the irritant. Hyaluronidase also prevented the gross edema produced by the oil-soluble irritant, but did not facilitate absorption. The dispersion of oil droplets by hyaluronidase caused extensive capillary injury with early fibrinous exudation and hemorrhage resulting in a barrier to further spread of the lesion. In non-hyaluronidase treated areas the early inflammatory response was milder and extravasation occurred later. In 72 hours the size of the involved areas was greater than in the hyaluronidase-treated areas.

Cardiovascular and serum potassium changes in fatal hyperthermia. L. I. GOLDBERG*, H. K. EZEEL* AND R. P. WALTON. *Depts. of Pharmacology and Chemistry, Med. College of South Carolina, Charleston.*

Fatal hyperthermia was produced in dogs by externally applied heat and by i.v. infusions of dicumarol (30-50 mg/kg.) and dinitrophenol (10 mg/kg.). All experiments were conducted under barbiturate anesthesia with vagotomy and positive pressure respiration with oxygen. In several particulars the course with external heat and with calorigenic drugs was closely similar. In both cases, ECG recordings were characteristic of hyperpotassemia. Heightening of the T wave occurred at average K levels of about 24 mg. % and rectosigmoid temperatures of about 40°C; similarly, S-A block developed at about 35 mg.% and 44°C; fatal termination followed shortly thereafter. As was demonstrated previously (*Federa-*

tion Proc. 8: 342, 1949; *Am. J. Physiol.*, in press), contractile force under these conditions ordinarily increased until near terminal stages. Progressive fall in arterial diastolic pressure levels (determined by Statham transducers) usually began at about 42.5°C. and was preceded by pulse contour changes with shortened run-off intervals. In most cases, diastolic pressure levels were below 30 mm.Hg when S-A block developed. Venous O_2 levels decreased much more rapidly with the calorigenic agents than with external heat. This reflects the known differences in metabolic rates with these two types of hyperthermia and indicates that the serum K levels were more related to body temperatures than to metabolic rate. Serum K levels were not clearly related to the rate of development of hyperthermia. Tromexan (65–100 mg/kg., i.v.) produced hyperthermia but, with less consistency, at slower rates and with severe initial hypotension.

Cutaneous and intravenous toxicity of 'endothal' disodium-3, 6-endoxohexahydrophthalic acid). FRANZ GOLDSTEIN (introduced by CHARLES M. GRUBER). *Div. of Industrial Medicine, Jefferson Med. College, Philadelphia, Pa.*

Endothal, a defoliant and herbicide, has been subjected to toxicity tests by both the Food and Drug Administration (SRENSEK AND WOODARD, *Federation Proc.* 10: 337, 1951) and the abovementioned Division. The cutaneous application of 1% endothal to the unbroken skin of rabbits produced no lesions, but the same solution applied to the scarified skin produced mild skin lesions. Application of more concentrated solutions (10% and 20%) and of pure powdered endothal to the intact or scarified skin resulted in more severe skin lesions with a tendency to necrotic changes. Death from absorption occurred in the cases showing the most severe skin lesions. The intravenous administration of 5 mg/kg. proved to be potentially fatal in both dog and rabbit, while doses of 10 mg/kg. proved invariably fatal. The magnitude of the lethal intravenous dose found in these experiments is in agreement with that reported by Srensek and Woodard. These investigators attributed death of the animals to respiratory failure. The studies referred to in this report tend to incriminate the heart as the primary organ of failure, as evidenced by symptomatology, pathological findings, electrocardiograms (evaluated by Dr. Charles W. Semisch, III), and recordings of blood pressure and respiration taken under anesthesia. Preliminary pharmacodynamic studies (done in collaboration with O. J. Inashima, Department of Pharmacology) have demonstrated a depressing effect of endothal in dilutions up to 1:20000 on the isolated frog ventricle and the isolated rabbit auricle. It remains to be determined by further pharmacologic studies whether this action on the heart is truly specific.

Mechanism of action of cortisone in experimental hypersensitivity. ANDRES GOTH, JAMES HOLMAN* AND JOHN H. COPENHAVER.* *Dept. of Pharmacology, Southwestern Med. School of the Univ. of Texas, Dallas.*

It has been shown in this laboratory (*Proc. Soc. Exper. Biol. & Med.*, in press) that cortisone exerts an effect on Tween hypersensitivity in male dogs. Whereas cortisone does not prevent histamine liberation following a single injection of Tween 20, it decreases or prevents the effect of a second Tween injection given 24 or 48 hours later. These experimental results suggested that cortisone prevents the reaccumulation of histamine following its liberation by Tween. This work has been extended to other experimental situations in which histamine is known to be released, such as anaphylaxis in the guinea pig and the response of the human skin to injections of RO2-2222. Twenty-four guinea pigs were sensitized to 4 different antigens intraperitoneally. Two weeks later the animals were divided into 2 groups, one treated with cortisone, the other left untreated. The 4 antigens were injected intraperitoneally into all guinea pigs at 24-hour intervals under Benadryl protection. Following the 4th antigen injection, all guinea pigs were passively sensitized to a pneumococcal polysaccharide. Twenty-four hours later, all animals were shocked with the polysaccharide. All untreated controls died, whereas 45% of the cortisone treated guinea pigs survived. The experiments with RO2-2222 are reported separately. Studies on the effect of cortisone on the histamine content of tissues are in progress.

Influence of certain ganglionic blocking agents and veratramine upon epinephrine and norepinephrine induced tachycardia in the isolated feline heart. JAMES GOURZIS*, HAROLD COOMBS*, ANNE CAMERON* AND A. J. PLUMMER. *Research Labs., Ciba Pharmaceutical Products, Inc., Summit, N. J.*

The tachycardia produced by adrenergic blocking drugs in the intact animal has been diminished by ganglionic blocking agents, (A. J. PLUMMER, W. B. REITZE AND F. F. YONKMAN, *Arch. Internat. pharmacodyn.*, in press). The theory that the causative factor was excess circulating epinephrine led to an attempt to block the cardioaccelerator effects of epinephrine and norepinephrine in the isolated feline heart by means of such ganglionic blockaders. The Langendorff technique as modified by Anderson and Craver (*J. Pharmacol. & Exper. Therap.* 93: 135, 1948) was employed. Extensive controls were performed using arbitrary dosages of L-epinephrine and DL-norepinephrine,

alone or alternately, which produced a definite tachycardia without secondary depression of the myocardium. Selected concentrations of several preparations were then perfused and the effect on epinephrine and norepinephrine tachycardia noted. The following drugs, in concentrations of 50 to 100 μg/ml. of perfusion fluid, gave evidence of reducing the cardioaccelerator effects of both epinephrine and norepinephrine: SU-1194, BA-9295 (Pendiomide) SU-1845, and SU-1792. The effects of other ganglionic blocking agents and veratramine will also be presented.

Action of sodium fluoroacetate on renal excretion of para-amino-hippurate. GILBERT GRAHAM* AND ALFRED FARAH. *Dept. of Pharmacology, New York State College of Medicine, Syracuse.*

In the intact anesthetized dog NaFAc in a dosage of 1.0–1.5 mg/kg. produces a 90–100% reduction of PAH Tm. The effect of NaFAc can be reduced by infusing dogs with sodium acetate. Sodium bicarbonate has no protective action. Self depression of PAH Tm at high PAH loads can be readily reversed with NaAc. NaFAc in a dosage of 1 mg/kg. prevents this effect of NaAc. PAH Tm once depressed by NaFAc cannot be increased either by sodium acetate or monoacetine. NaFAc in the dosages used produced a reduction in blood flow and glomerular filtration. *In vitro* experiments have shown that dog kidney cortex slices can concentrate PAH in a manner similar to rabbit kidney slices (CROSS AND TAGGART, *Am. J. Physiol.* 161: 181, 1950). Kidney slices from fluoroacetate poisoned dogs show mainly a defect in the ability of sodium acetate to stimulate the uptake of PAH. *In vitro* studies show that PAH uptake by kidney slices is depressed by concentrations of sodium fluoroacetate which have none or minimal effects on the oxygen consumption of kidney cortex slices. NaAc markedly protects kidney slices against the effects of NaFAc. A study of the actions of sodium fluoroacetate on other kidney functions is now in progress.

Distribution of histamine among blood elements. HELEN TREDWAY GRAHAM, FRANCES WHEELWRIGHT*, HAVNER H. PARISH, JR.*, ANCEL R. MARKS* AND OLIVER H. LOWRY. *Depts. of Pharmacology and Medicine, Washington Univ. School of Medicine, St. Louis, Mo.*

By use of the micro dinitrofluorobenzene method for histamine measurement, combined with differential cell counts, the basophils have been found to contain approximately 50 times more histamine than any other blood cell type. Because the granulocytes were known to contain much of the blood histamine, and because histamine and the basophil count both increase in chronic myelogenous leukemia, partial separation of the varieties of leucocytes was effected by centrifugation, and histamine measurements and differential leukocyte counts were performed on successive layers. From one case of chronic myelogenous leukemia (15% basophils), layers were obtained with 9-fold difference in basophil content (3 to 27%). The relation of the histamine content to the numbers of each cell type was studied by statistical analysis *1*) in these layers as well as in *2*) blood from normal persons and *3*) from patients with various blood disorders. In each instance, the basophils were much the richest in histamine. The blood histamine of 21 normal people averaged 122 μg/l. (S.D. 56). The histamine per liter of each element and the percentage contribution to the total blood histamine are approximately as follows: plasma, 5 μg., 4%; erythrocytes, 57 μg., 20%; platelets, 1200 μg., 3%; neutrophils, 10,000 μg., 50%; basophils, 5000,000 μg., 15%; eosinophils, not over 10,000 μg. and 1%. Lymphocytes and monocytes probably contain very little histamine. In the chronic myelogenous leukemia patient referred to, the blood histamine was 12 mg/l., of which at least 90% was calculated to be in the basophils.

Factors affecting potentiation of barbiturate action by tetraethylthiuram disulfide. W. DONALD GRAHAM, H. TEED AND M. G. ALLMARK (introduced by F. C. LU). *Food and Drug Labs., Dept. of Natl. Health and Welfare, Ottawa, Canada.*

Previous work has shown that tetraethylthiuram disulfide (TETD) will potentiate barbiturate action in rats and guinea pigs (*J. Pharmacol.* 3: 497, 1951). Experiments have been conducted to determine whether or not sodium ascorbate or reduced glutathione would decrease the degree of barbiturate-potentiation caused by fixed doses of TETD. At the dosage range and in the manner employed in this work, neither of these compounds was effective. In contrast to the results of Giarman *et al.* with mice (*Science* 114: 35, 1951) repeated administration of TETD to rats did not intensify the effect on barbiturate anesthesia beyond that achieved by a single dose.

Spontaneous and therapeutically induced alterations of serum complement in nephrotic syndrome. FRANK GRAIG, KURT LANGE, LAURENCE SLOBODY AND JACOB OBERMAN (introduced by M. G. MULINOS). *Depts. of Medicine and Pediatrics, New York Med. College, New York City.*

Serum complement is abnormally low in the nephrotic syndrome. This is not due to loss of complement into the urine or anticomplementary substances present in the blood. It is probably the result of a complement binding antigen-antibody reaction which is the basis of glomerulonephritis and so-called pure nephrosis. Adrenocorticotropin (ACTH) and Nitrogen Mustard therapy does not

change the serum complement level in normal individuals. Treatment of nephrotic patients with these compounds results in a rise of complement to or toward normal 24–48 hours before the onset of diuresis. Spontaneous diuresis shows the same change in complement. Reaccumulation of nephrotic edema is preceded by a fall in complement. Small maintenance doses following a successful course of ACTH often do not prevent clinical and immunologic relapse. High doses of ACTH repeated in short courses at short intervals seem to yield much better long-lasting results.

Intravenous infusion studies in dogs with epinephrine and L-arterenol. JESSE I. GRANT* AND A. M. LANDS. *Pharmacology Section, Sterling-Winthrop Research Inst., Rensselaer, N. Y.*

Dogs were anesthetized with pentothal and barbital. Systolic and diastolic blood pressure was recorded kymographically with an Anderson glass capsule manometer; respiration with a Marey tambour, and the femoral vein cannulated for infusion of drugs. Two micrograms (as base) of each compound dissolved in 2 ml. of 0.85% saline containing 0.1% sodium acetone bisulfite as a preservative were infused/kilogram/minute (Peterson-Graubard flowrator). Analysis of the measurements covering the 10 minute recorded period shows L-arterenol (nor-epinephrine) 1.43, 1.54 and 1.47 times more effective in elevating systolic, diastolic and mean arterial pressure respectively. L-Arterenol increased pulse pressure to a greater extent than epinephrine (synthetic levo isomer) in 8/13 experiments; epinephrine to a greater extent in 4/13 experiments; and in 1/13 experiments pulse pressure was increased to the same degree by both sympathomimetic amines. In 4/8 experiments L-arterenol produced greater cardiac output than epinephrine; in 2/8 experiments epinephrine gave greater cardiac output; and in 2/8 experiments the increased cardiac output was of the same order of magnitude for both compounds. In 5/9 experiments where epinephrine had practically no effect on heart rate, L-arterenol caused marked bradycardia in 3 and tachycardia in 2 of the unatropinized dogs; in 2/9 experiments bradycardia produced by L-arterenol was more than twice that produced by epinephrine; in 1/9 experiments epinephrine produced marked bradycardia, L-arterenol marked tachycardia; and in 1/9 experiments both compounds showed equal bradycardia. In 3/9 experiments L-arterenol caused tachypnea and epinephrine bradypnea; in 2/9 experiments where L-arterenol had no effect on respiration, epinephrine caused tachypnea; in 2/9 experiments bradypnea response to L-arterenol was 2–3 times greater than bradypnea response to epinephrine; in 1/9 experiments bradypnea was the same for both drugs; finally in 1/9 experiments where epinephrine caused mild tachypnea, L-arterenol caused marked bradypnea. These data indicate that the differences between L-arterenol and synthetic levo epinephrine are quantitative rather than qualitative in nature.

Factors influencing post-DCA hypertension. D. M. GREEN, F. J. SAUNDERS* AND NANCY WAHLGREN.* *Div. of Biological Research, G. D. Searle & Co., Chicago, Ill.*

Rats given 20 mg. desoxycorticosterone acetate (DCA) implants and high sodium intake developed severe hypertension which persisted after the pellet had been absorbed. The degree of post-DCA hypertension was not influenced by the following procedures or treatments: castration, adrenalectomy, nephrectomy, low sodium diet, low potassium diet, ACTH or cortisone. The blood pressure was reduced by propylthiouracil. Thyroid-parathyroidectomy was followed by a fall not different from that produced by propylthiouracil. Thyroxin injections significantly increased the hypertension. A small rise was produced by large doses of calciferol. Hypophysectomy was followed by a fall in pressure which progressed downward toward normal over a period of weeks but was associated with a high mortality. Dibenamine produced a moderate and transient fall in pressure. Barbiturate anesthesia was followed by a prompt, temporary reduction to normal and a post-anesthetic return to the previous pressure level.

Effect of natural metabolites on the in vitro activity of certain antimalarials. JOSEPH GREENBERG, EDWARD S. JOSEPHSON AND D. JANE TAYLOR (introduced by S. M. ROSENTHAL). *Natl. Microbiological Inst., Natl. Insts. of Health, Bethesda, Md.*

Experiments were carried out to determine the mode of action of certain antimalarials by examining the effect of natural metabolites on their *in vitro* activity against the erythrocytic forms of *Plasmodium gallinaceum*. Quinacrine, 2-(*p*-chlorophenyl) guanidino-4-(β-diethylaminoethylamino)-6-methylpyrimidine and 2-(*p*-chloroanilino)-4-(*p*-dimethylaminoethylamino)-6-methylpyrimidine which compete with riboflavin in *Lactobacillus casei* were tested. These compounds were found to be plasmodicidal *in vitro* in concentrations approximating those found *in vivo* in the blood of adequately treated chicks. This is in contrast to the inactivity *in vitro* of 2,4-diamino-5-(*p*-chlorophenyl)-6-ethylpyrimidine and its analogues. Riboflavin failed to reverse the inhibition produced by any of the compounds when added in amounts up to 10 times the concentration of the inhibitors. In addition, riboflavin phosphate, adenine, adenosine, adenylic acid, glucose-6-phos-

phate and glucose-1-phosphate failed to reverse the plasmodicidal effect of quinacrine.

Bioassay of mercurial diuretics by oral administration in cardiac patients with congestive failure. THEODORE GREINER, HARRY GOLD, LEON WARSHAW*, FRANK PALUMBO*, JOHN WEAVER*, SYDNEY MATHES* AND RAYMOND MARSH.* *Dept. of Pharmacology, Cornell Univ. Med. College, Cardiovascular Research Unit, Beth Israel Hosp., and Cardiology Service, Hosp. for Joint Diseases, New York City.*

In a recent paper which we published in the *Journal of Pharmacology*, we outlined a method for the bioassay of mercurial diuretics in ambulant patients with congestive failure, which complied with the standards generally acceptable for bioassay in animals in the U. S. Pharmacopeia. We have now applied this method to 3 mercurial diuretics intended for oral administration, and the results of this assay constitute the subject of the present report. We were provided with reports of observations on dogs, as well as of therapeutic trials in man, suggesting that these compounds were of similar potency. The details of the method are as follows: the diuretic response is the loss of weight 24 hours after the dose is administered. Additional weights taken in 48 hours demonstrated that even for oral diuretics the 24-hour weight measures essentially all the diuresis. The potency is stated in terms of a standard (mercuhydrin by intramuscular injection) and the precision calculated as confidence limits. The results of this assay showed that one of these compounds, namely, 3-chloromercuri-2-methoxypropylurea, by oral administration possessed three-fourths the diuretic action of the standard. This marks an unusual advance in oral mercurial therapy, for all other compounds that have so far been tested come no nearer than one-fourth the diuretic potency of the standard. The results also show the importance of the method of bioassay of mercurial diuretics.

Absorption, distribution and activity of Tromexan. CHARLES M. GRUBER, JR., KWANG SOO LEE* AND FREDRIC RIEDERS.* *Dept. of Pharmacology, Jefferson Med. College, Philadelphia, Pa.*

Large single oral doses of Tromexan were given to dogs. Plasma levels were measured spectrophotometrically by the method of Rieders and Gruber, Jr. Prothrombin times were measured by the one-stage Quick method using human brain thromboplastin. Maximum Tromexan concentrations occurred in the plasma 8–12 hours after administration and maximum prothrombin times occurred 2–3 days later. No relationship was found to exist between the time of occurrence, the degree of the responses, and/or the area enclosed by the two curves. Statistically significant differences in the maximum plasma concentrations and the time of occurrence of these maxima were found on comparing fed dogs given 40 mg/kg. and fasted dogs given 50 mg/kg. These results suggest the presence of a stabilized absorption and storage mechanism which is modified by diet and/or dose. The thoracic duct was cannulated 18–20 hours prior to administration of Tromexan. The systemic blood specimen at every time interval contained a greater concentration than did the thoracic lymph. This indicates transportation primarily through the portal circulation and suggests unequal distribution in the extra-cellular fluids. Approximately 50 hours after administering Tromexan, 37.5 mg. of Menadione sodium bisulfite per kilogram body weight were given intravenously. All of the animals obtained a maximum reduction in the prothrombin time in 1 hour. This effect was maintained for about 6 hours. It is doubtful if an effect occurring with such rapidity can be explained on the basis of prothrombin resynthesis in the liver.

Mechanism of hypotensive action of 'Veraloid'. CARL C. GRUHZIT* AND G. K. MOE. *Dept. of Physiology, State Univ. of New York Med. Center, Syracuse.*

The effect of 'Veraloid,' a biologically standardized preparation of Veratrum veride, was studied in dogs under thiopental-barbital anesthesia. Single injections of 5 mcg/kg. or infusions of one mcg/kg/min. produced bradycardia and hypotension in the intact animal. In addition, Veraloid markedly reduced or abolished the pressor effect of carotid clamping and the fall in blood pressure produced by tetraethylammonium chloride (TEA). Unlike dihydroergocornine, however, it did not prevent that component of the asphyxial pressor response which persists in the presence of TEA. Atropinization or bilateral cervical vagotomy reduced the cardiac slowing but failed to alter significantly the hypotensive effect of Veraloid. Following section of Hering's nerve Veraloid no longer produced a fall in blood pressure, and in some instances even raised the pressure slightly. In several experiments bilateral denervation of the carotid sinuses was accomplished by removal of the nerve plexi from the surfaces of the carotid sinuses and adjoining regions. The denervation was judged to be complete when no rise in pressure was produced by clamping the common carotid arteries. In these cases the hypotensive action of Veraloid was abolished. Since the carotid bodies were still intact, as determined by the respiratory response to intravenous injections of sodium cyanide, it seems probable that the hypotensive action of Veraloid is due in considerable measure to the stimulation of afferents in the carotid sinus region.

Potency and persistence of effect of several digitalis preparations in pigeons. HARVEY B. HAAG AND SALLY WOMACK.* *Dept. of Pharmacology, Med. College of Virginia, Richmond.*

The current USP XIV (official November 1, 1950) specifies the use of pigeons in the biological standardization of digitalis. In the present investigation using pigeons, it was found that of 6 commercial digitalis leaf preparations studied 5 were in satisfactory agreement with USP requirements, while one was not. The USP digitalis unitage equivalent for reference digitoxin was 0.44 mg.; for reference digoxin, 0.33 mg. Persistence studies were made by injecting 60% of the USP unitage equivalent and, by titration at certain intervals, establishing what percentage of the initially administered digitalis effectiveness then remained. Two series of such experiments were done. In the first the various digitalis preparations were not studied concurrently and the results showed appreciable differences between commercial samples and the reference leaf. In the second series the samples were studied concurrently and the results then revealed no differences in persistence of effect. Furthermore while the reference leaf manifested significant persistence after 10 days in the first experiments, it evidenced none whatsoever after 5 days in the second series of tests. Based on average values neither digoxin nor digitoxin showed significant persistence after 5 days, although individual pigeons did demonstrate a marked effect. These results raise a question as to the suitability of pigeons for studies concerned with persistence of digitalis effectiveness. (Aided by a grant from the Ciba Pharmaceutical Products, Inc.)

Oral toxicity of 2 2′ methylene bis (4 methyl-6-tertiary-butylphenol) (2246). ERNEST C. HAGAN (introduced by JOHN H. DRAIZE). *Div. of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*

Compound 2246 has been suggested for use in the prevention of rancidity of fats and oils. Chemically it possesses certain structural similarities to the anticoagulants Dicoumarol and Warfarin. We have studied the acute toxicity of 2246 by oral administration of corn oil suspensions to rats, guinea pigs, and mice, and by dietary feeding to rats with special emphasis on prothrombin times in rats. The LD_{50} for rats is 6500 mg/kg. Preliminary data indicate guinea pigs and mice have a similar susceptibility. Symptoms of acute poisoning are listlessness, ruffled coats, and hemorrhage about the nose. Deaths are often delayed several days after dosage. Prothrombin times of plasmas of rats dying these delayed deaths are within the normal range. Rat dietary concentrations of 1.0 and 0.5% of 2246 cause deaths within 2 and 4 weeks respectively, preceded by gross symptoms consisting of pallor and listlessness. Extensive subcutaneous and internal hemorrhages are observed at autopsy. Plasma prothrombin times of these subacutely poisoned rats are 3–15 times greater than the 16–22 seconds obtained for the untreated control rats. (Plasmas of untreated control rats serially diluted with saline to concentrations as low as 20% give normal prothrombin times.) Rats fed the 0.5% concentration show increase in prothrombin times prior to the occurrence of symptoms. Apparently deaths resulting from subacute feeding have as their principal cause the hemorrhage resulting from prothrombin inhibition.

Ineffectiveness of flavonoid compounds in reducing radiation mortality. THOMAS J. HALEY AND SAMUEL MANN.* *Atomic Energy Project, Univ. of California School of Medicine, Los Angeles.*

Confusion exists regarding the ability of flavonoid compounds to counteract irradiation injury. Sokoloff *et al.* (*Science* 112: 112: 1950) and Griffith *et al.* (*Proc. Soc. Exper. Biol. & Med.* 64: 331: 1947) reported flavonoids beneficial in rats while Kohn *et al.* (*Report AECD* 2176, June 7, 1948) reported no effect in rats and Cronkite *et al.* (*Proc. Soc. Exper. Biol. & Med.* 70: 125: 1949) no effect in mice. These divergent viewpoints led us to investigate the effect of the flavonoids in guinea pigs subjected to 200 r acute whole body roentgen ray irradiation. The drugs were administered intraperitoneally in the following doses: rutin, 250 mg/kg.; hesperidin methyl chalcone, 100 mg/kg.; naringin, 75 mg/kg.; hesperidin, 25 mg/kg. The animals were injected either 10 days pre- or post-irradiation and observed for 1 month. Irradiated animals all showed definite symptoms of irradiation injury and the total mortality figures were approximately the same for both the medicated and non-medicated groups. However, both the pre- and post-irradiation flavonoid groups showed increased rates of mortality over those of the non-medicated control groups. No deaths occurred in the non-irradiated medicated or saline injected controls during the experimental period. The ineffectiveness observed was not due to ascorbic acid deficiency (scurvy) because the animals were maintained on a high ascorbic acid intake prior to and after irradiation. Therefore it is concluded that these flavonoid compounds are not beneficial in radiation injury and can be highly detrimental to the animal. (Based on work performed under Contract No. AT-04-1-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.)

Phenoxyalkyltrimethylammonium derivatives with pressor activity. W. E. Hambourger, Laurie Ann Murray* and Deane A. Houlahan.* *Div. of Biological Research, G. D. Searle & Co., Chicago, Ill.*

A series of substituted phenoxyalkyltrialkylammonium derivatives, prepared in our laboratories by Clinton A. Dornfeld, was tested in the cat for sympathetic ganglion blockade. Two exceptional members of the series were found: SC-3952, the o-phenyl, and SC-3954, the o-cyclohexyl, derivatives of phenoxyethyltrimethylammonium iodide. Both produced a sharp rise in blood pressure with some secondary fall, similar to the epinephrine response. Also like epinephrine, they produced a brief contraction of the nictitating membrane. Dibenamine abolished these pressor responses and sometimes produced a reversal; it also diminished or abolished the contractions of the nictitating membrane. Priscoline similarly reduced the rise in blood pressure. The pressor effect was lost when the phenyl and cyclohexyl substituents were shifted from the ortho to the para position. The analogous triethylammonium halides likewise failed to raise blood pressure. The hydrochloride salts of the tertiary bases corresponding to SC-3952 and SC-3954 were also devoid of pressor activity.

Papaverine analogs III. Some 3,4-dihydroisoquinolines as coronary vasodilators. Calvin Hanna and James Shutt (introduced by E. G. Gross). *Dept. of Pharmacology, State Univ. of Iowa, Iowa City.*

In an effort to determine the structural features of the papaverine molecule most essential for coronary dilator action a group of 3,4-dihydroisoquinolines has been prepared. These compounds were quantitatively tested by the method of Winder *et al.* (*J. Pharmacol. Exper. Therap.* 100: 482, 1950). Three-four-dihydropapaverine was found to have 75% of the activity of papaverine while 1-benzyl-6,7-dimethoxy, 1-benzyl-6,7-methylenedioxy- and 1-methyl-6,7-dimethoxy-3,4-dihydroisoquinolines has 33%, 30% and 20% of the activity, respectively. The methyl iodides of 3,4-dihydropapaverine and papaverine have no dilator action. One-(2,5-dimethoxybenzyl)-6,7-dimethoxy-3,4-dihydroisoquinoline was prepared in an attempt to relate the *ortho* methoxy groups of papaverine to the corresponding *para* methoxy groups in khellin. This compound is equal in potency to and is somewhat longer acting than papaverine. One way of interpreting the similar vasodilator action of these compounds would be to postulate that they act following demethylation. Papaveroline, the demethylated product of papaverine, was found to have 65% of the activity of papaverine, following which papaverine was inactive. The fact that papaveroline may block receptors sensitive to papaverine supports the idea that papaverine acts following demethylation.

Relation of copper to cardiac glycoside action. Harold Hardman,* Donald R. Bennett,* John F. Palmer,* Robert Parry* and Maynard B. Chenoweth. *Depts. of Pharmacology and Chemistry, Univ. of Michigan, Ann Arbor.*

It was reported (*J. Pharmacol. & Exper. Therap.* 103:340) that ouabain forms a complex with copper having its absorption maxima in the ultraviolet and infra-red spectra and that copper influences the toxicity of cardiac glycosides to animals. If copper chelation is a basic mechanism in the action of these glycosides, then the administration of chelating agents or compounds that react with copper might affect the positive inotropic action of ouabain. The results of this study using the cat papillary muscle preparation fall into two classes. *Class I:* compounds that stimulate and/or potentiate the action of ouabain. *Class II:* compounds that block the action of ouabain. *Class I* includes ethylene diamine and the disodium salt of ethylene diamine tetracetic acid which certainly stabilize the cupric form and dimercaprol and dehydroacetic acid which probably stabilize the cupric form. *Class II* includes sodium iodide and sodium thiocyanate which certainly stabilize the cuprous form and thyroxine and thiopental which probably stablize the cuprous form. Extensions of this and other work on the problem will be reported.

Estimation of posterior pituitary activity in commercial preparations of ACTH. William D. Harkness, Stanley E. Srensek and Geoffrey Woodard (introduced by Bert J. Vos). *Div. of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*

The commercially practiced methods of manufacture of ACTH would likely result in its contamination with the principles of the posterior pituitary. All samples tested by us thus far have shown measurable posterior pituitary activity. The chicken blood pressure, guinea pig uterus contraction and antidiuresis in rats were used for estimation of this activity. With the chicken method the most frequent observation was the noncharacteristic fall in blood pressure following injection of ACTH. Its injection produced a slower fall in blood pressure than posterior pituitary and a more delayed return to normal. Variability in normal base line between doses made accurate measurement difficult and ACTH appeared to reduce responses to subsequent doses of posterior pituitary. The character of the uterine response to posterior pituitary does not seem to be signifi-

cantly altered by the addition of ACTH except for the contamination already present in the ACTH. Aside from the familiar technical difficulties the uterine response seems useful for estimation of the contamination. There has been fair agreement among the 3 assay procedures. The uterine method is the most sensitive and requires the least material for an assay. The rat antidiuretic method is intermediate, while the chicken blood pressure is least sensitive and requires the most material.

Anticonvulsant action of Hibicon (N-benzyl-B-chloropropionamide). B. K. HARNED, RAYMOND W. CUNNINGHAM, FRANK H. SMITH,* MARY C. CLARK,* MARGARET KANE.* *Lederle Labs. Div., American Cyanamid Co., Pearl River, N. Y.*

Hibicon is an effective antiepileptic with a chemical nucleus new in the field of anticonvulsants. An extensive comparison of the action of this compound with that of Dilantin in man and in experimental animals indicates that the anticonvulsant potentials of the 2 drugs are of the same order but the side reactions, especially anorexia and muscular stiffness, are less after Hibicon. Anticonvulsant potency was evaluated in rats, cats and mice on the basis of 4 methods: *1*) the maximal electroshock seizure test in rats; *2*) the Putnam-Merritt electroshock test in cats and in rats; *3*) the intravenous Metrazol test in mice; and *4*) the audiogenic-Metrazol test in rats. The acute toxicity of Hibicon is low; in rats the oral LD_{50} is 3200 mg/kg. with 19/20 limits 3098–3306. Rats fed daily doses of 1500 mg/kg/day for 1 year showed no evidence of chronic toxicity except a depression of 25% in growth. For this species the oral Effective $Dose_{50}$ by the maximal electroshock seizure test is 46 mg/kg. with 19/20 limits 30–71. In rats anticonvulsant activity of an asymptomatic oral dose can be detected within 30 minutes. Maximal activity is attained in 2 hours and continues through the 6th hour. Anticonvulsant activity is present for 12 hours. The liver and also the kidneys are important factors in determining the duration of action of Hibicon. This drug is not sedative, antispasmodic, oxytocic, miotic, mydriatic analgesic or histaminic. Large intraperitoneal doses of Hibicon produce a moderate decrease in blood pressure in anesthetized animals; however, the vasomotor responses to epinephrine, acetycholine and faradic stimulation of the vagus are not modified.

Comparison of rate of uptake of radioactive thyroxine by the hypophysis in the normal, hypothyroid and hypermetabolic rabbit. EDWARD O. HARPER,* PAUL A. MATTIS AND J. W. BOEHNE.* *Depts. of Psychiatry and Pharmacology, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

Horeau and Sue in 1945 demonstrated the presence of radioactive thyroxine in the hypothalamus and hypophysis of rabbits. In previous studies we have shown that the tuber cinereum and pituitary of normal animals selectively concentrate thyroxine; and that although the concentration in the pituitary exceeds that attained in the tuber the absorption curves of the 2 tissues are similar. In the thyroidectomized rabbit the maximum concentration of thyroxine in the pituitary is greater than in the normal animal although the rate of uptake is slower. In this study 15 normal, virgin female rabbits were made hypermetabolic by the subcutaneous administration of commercial thyroxine (500 μg/kg). Three or 4 days following administration, when a definite hypermetabolic state was found to exist, 10 μg/kg. of radioactive thyroxine were given intravenously to each rabbit. Three animals were sacrificed for the determination of tissue concentrations of thyroxine at 1, 2, 4, 12 and 24 hours following the administration of the labeled hormone. The tissues were digested and activity determined by a Geiger-Mueller counter. The concentration is expressed by the ratio

$$\frac{dis/min/mg/organ\ wt/.}{dis/min/mg/body\ wt/.}$$

The concentration and rate of absorption of radioactive thyroxine by the pituitary of these animals is significantly less than in normal and thyroidectomized rabbits. Radioautographic analysis of the tissue extract, using partition chromatographic techniques, revealed that the major portion of the radioactivity in the pituitary was due to labeled thyroxine. Some of the implications of these findings will be discussed.

Interrelation of autonomic ganglionic effects of hexamethylene-bis-trimethylammonium (C6) and tetraethylammonium (TEA). STEWART C. HARVEY, JOHN L. BAUKOL,* GWYLLUM BLASER,* FRED W. HEATON* AND LAMAR H. STEWART.* *Dept. of Pharmacology, Univ. of Utah, Salt Lake City.*

The peculiar neuromuscular interrelation of *d*-tubocurarine and decamethylene-bis-trimethylammonium (C10), and the analogous effects of tetraethylammonium (TEA) on autonomic ganglia and *d*-tubocurarine on the neuromuscular junction suggested a study of the interrelation of TEA and hexamethylene-bis-trimethylammonium (C6) on the superior cervical ganglion in cats. Contraction of the nictitating membrane induced by supramaximal 3/second preganglionic stimulation was recorded as a function of synaptic transmission. Dose-inhibition curves of TEA, C6 and combined TEA-C6 were determined. Analysis of the curves revealed true and marked potentiation when TEA is administered immediately following C6. C6 ad-

ministered during constant infusion of TEA exerted its expected degree of inhibition (in contrast to antagonism by *d*-tubocurarine prior to C10 at the neuromuscular junction). Interruption of the TEA infusion resulted in a C6 residual effect exactly equal to control inhibitory effect of C6, and the slope and intercept of the postinfusion and control recovery curves were identical. This suggests, along with the observed potentiation, that the 2 drugs do not act at the same synaptic locus. In a single experiment, constant infusion of minute amounts of TEA (0.0047 mg/kg/min.) repeatedly resulted in an augmentation of nictitating membrane response, which fact suggests that TEA may possess weak nicotinic stimulant properties. Further study of this property is in progress. (Supported by a grant from the Givaudan Corporation.)

Adapted method for observing eosinophil changes in intact rats. ARTHUR E. HEMING, MARY F. SAX AND DORSEY E. HOLTKAMP (introduced by EDWIN J. FELLOWS). *Research and Development Div., Smith, Kline and French Labs., Philadelphia, Pa.*

Eosinopenia is well established as a measure of the alarm reaction (*J. Clin. Endocrinol.* 10: 187, 1950). However, it has been difficult to study the alarm reaction in adult intact rats by following the eosinophil level because either the individual rat's blood eosinophil count is too low to permit accurate appraisal, or the experimental techniques themselves produced an eosinopenia. These difficulties have been overcome by the methods here described. Tail-blood taken immediately before, and 4 hours after the intraperitoneal injection of the test substance, is diluted 1:20 with Hinkleman's fluid (*Am. J. M. Sc.* 221:121, 1951). Eosinophil counts are made in a 0.2-mm.-deep chamber with Fuchs-Rosenthal rulings. Four rulings are counted for low counts. Rats are handled with care prior to and during the test period. All rats are screened initially to select those which show: *1*) either an eosinophilic response or an eosinopenic response of <15% to a 5% glucose injection (1 cc/kg.); *2*) an eosinopenic response of >25% when epinephrine (0.25 mg/kg.) is injected; and *3*) an initial count >62 eosinophils/cu.mm. Sixty of 99 rats examined were found suitable for use by these criteria. From these 60 rats, 3 groups of 12 each, were selected randomly, and given, respectively: histamine (10 mg/kg.), formaldehyde (25 mg/kg.) and 0.9% saline (1 cc/kg.). The saline injection like the glucose, failed to produce a reaction; whereas, the histamine and formaldehyde produced an eosinopenic response comparable to that obtained with epinephrine.

Curare compounds. XIII. Fate of carbon-14 labeled D-tubocurarine dimethylether in guinea pigs. D. A. HERRING* AND DAVID F. MARSH. *Dept. of Pharmacology, West Virginia Univ. School of Medicine, Morgantown.*

D-Tubocurarine dimethylether iodide labeled with radioactive carbon atoms in 2 methoxyl groups and 2 nitrogen methyl groups was prepared. This material has an activity of 1,400 counts/min/μg. with our present counting equipment. In addition, some experiments were carried out with D-tubocurarine dimethylether iodide labeled in 2 methoxy groups and D-chondocurarine iodide labeled in 2 nitrogen methyl groups. Twelve male guinea pigs (Rockland) weighing 700–850 gm. were used in 3 experiments. Intravenous injection of 22 μg. of the dimethylether/kg. (22–26,000 cpm total) produces almost immediate head drop and partial skeletal muscle paralysis. The urine and feces were collected separately, dried *in vacuo*, extracted with methanol, plated, and counted to a maximum 14% error. Within 2 hours, 51–73% of the total activity is excreted in the urine and 67–91% within 24 hours. Intramuscular injection of 70 μg. of the dimethylether/kg. produces head drop in 10–13 minutes that lasts 16–24 minutes. Eighty-seven to 94% of the total injected radioactivity is found in the urine in 24 hours; these urine samples were pooled and paper chromatographed by a modification of Dutcher's method. At least 65% of the radioactivity is due to unchanged dimethylether; the balance is represented by a small amount of only 1 less mobile metabolite and indefinite losses. Similar experiments with D-chondocurarine (50 μg/kg.; 24–30,000 cpm) produces head drop within 11–24 minutes that lasts 16–24 minutes. Comparable total quantities of radioactive material are excreted in the urine although at a lower rate. Paper chromatography indicates about 35–40% as unchanged material and the presence of 3 or 4 metabolites. Less than 3% of either agent is excreted in the feces.

Adaptation of the uterine musculature of rabbits to vasopressin. JAMES G. HILTON* AND ROBERT A. WOODBURY. *Div. of Pharmacology, Univ. of Tennessee, Memphis.*

Experiments were conducted upon excised uterine horns of rabbits which previously had received 10 μg/animal diethylstilbestrol intramuscularly daily for 3 weeks and 5 U of vasopressin tannate intramuscularly on alternate days for the last 14 days (7 injections). By test in Tyrode bath solution the uterine responses to vasopressin were not materially different from those of control strips from animals receiving only diethylstilbestrol. Rabbits similarly treated which also received 0.5 mg/animal progesterone daily during the time of vasopressin tannate administrations showed a marked change in the influence of vasopressin on the uterus (1×10^{-5}, 1×10^{-4}, 1×10^{-3} ml. of vasopressin/ml. of bath). In these tests vasopressin caused much less increase in the height and rate of

contraction and occasionally produced a type of contraction which was longer in duration and which exhibited more complete relaxation. (This slower more cordinated form of contraction was apparent only at the lowest concentration of vasopressin.) Vasopressin actually decreased tone and rate of contractions in 14 and 29% of the sections, respectively, at the medium and large concentrations. The possible mechanism for this modification phenomenon appears to be one of tissue adaptation to the administered vasopressin.

Toxicity in rodents and anti-cholinesterase activity of organo-phosphorous compounds. C. H. HINE, E. R. SHACK,* J. K. KODAMA* AND M. S. MORSE.* *Div. of Pharmacology and Experimental Therapeutics, Univ. of California, San Francisco.*

The acute toxicity of 64 organic derivatives of phosphorus containing acids was determined in mice and rats following oral administration. The cholinesterase (Ch.E.) inhibitory properties of these agents were studied *in vitro* by the method of Michel (MICHEL, H. O. *J. Lab. & Clin. Med.* 34: 1564, 1949) against preparations of human red blood cells and rat brains. Evidence of Ch.E. inhibition was found in compounds of the phosphate and phosphonate type. No activity was observed in phosphinates, phosphine oxides, phosphites, phosphinites, and phosphines. The phosphonates [$(RO)_2O—\overset{\|}{\underset{P}{}}R'$] were slightly less active than were their phosphate homologues [$(RO)_2O—\overset{\|}{\underset{P}{}}OR'$]. *In vitro* Ch.E. inhibition in general paralleled mammalian toxicity, but there were notable exceptions. Greatest activity and toxicity were present when R's were ethyl. Substitution of a thio or amino group for the oxygen linkage decreased activity. Groups which increased the electrophilic activity of the OR′ or R′ chain enhanced activity.

Electrical activity of the small intestine and its modification by certain drugs. DUNCAN A. HOLADAY, HERBERT VOLK AND JULIAN MANDEL (introduced by E. M. PAPPER). *Depts. of Anesthesiology, Surgery and Radiology, Columbia Univ. College of Physicians and Surgeons, New York City.*

The small intestine of man, dog, cat and rabbit exhibits 2 types of electrical activity. Bursts of negative, spike-like action potentials, lasting approximately 60 msec., precede the appearance of visible contractions by ½ second. These are not unlike the action potentials of skeletal muscle and are interpreted as being similar in nature. Choline esters and anti-cholinesterases increase the frequency of these discharges, and parasympatholytic drugs and epinephrine suppress them. The second type of activity consists of rhythmic, 2- to 3-mv. fluctuations of the resting potential of the bowel at a frequency of 12–15/minute. The duration, magnitude and frequency of these waves are unaffected by the presence or absence of motor activity. The contour of the wave in duodenum and upper jejunum is more regular than in lower portions of the bowel. The wave form is most regular in the dog, and is less regular in the other species studied. It is generally unaffected by drugs administered intravenously, but may be altered by close intra-arterial injections. Initiation of spike potentials occurs only during the positive phase of the slow wave. Spike potentials are almost always absent during the negative phase. The responses of the intestine to certain drugs, together with an interpretation of the origin of the slow wave, will be discussed.

Toxicity of malathon (0,0-dimethyl dithiophosphate of diethyl mercaptosuccinate). EMILY G. HOLLAND,* LLOYD W. HAZLETON AND DOROTHY L. HANZAL. *Hazleton Labs., Falls Church, Va.*

The insecticide malathon (the approved common, nonproprietary name of a new member of the organic phosphate group) has been under extensive investigation in our laboratories for more than 2 years. It appears to be relatively nontoxic in warm-blooded animals. The acute oral LD_{50}'s of the 90% technical compound when administered in vegetable oil are: male mice, 885 mg/kg., and male rats, 480 mg/kg. Male and female rats on chronic toxicity studies have satisfactorily tolerated 1,000 parts/million in their diets for a period of 6 months. Dogs administered large oral or intravenous doses of malathon have survived. Dermal absorption has not appeared to be a toxicity problem in rabbits or guinea pigs receiving single or repeated applications. Malathon is a cholinesterase inhibitor but the pharmacological symptoms are of short duration. A purified 99+% form of malathon was found to be appreciably less toxic.

Effect of acetyl choline on the permeability to Na and K of isolated guinea pig hearts. W. C. HOLLAND, CLARA ELIZABETH DUNN* AND MARGARET E. GREIG. *Dept. of Pharmacology, Vanderbilt Univ. School of Medicine, Nashville, Tenn.*

We have found evidence, in isolated guinea pig hearts, of a correlation between permeability to Na and K and rate of acetylcholine metabolism. The effect of acethylcholine on the auricle differs in several respects from that on the ventricle. In a K-free medium acetylcholine increases the loss of K from both tissues, stops the auricle but has no effect on the ventricle, and depresses the ST segment of the EKG. In a medium containing 400 mg. KCl/l., acetylcholine causes both tissues to pick up K, the ventricle picking up 2–3 times as much/gm. of muscle as the auricle. Under these conditions, acetylcholine stops the auricle completely

and inhibits the ventricle temporarily (2–3 minutes). The T-wave of the EKG is elevated by acetylcholine. In a medium containing 800 mg. KCl/l., acetylcholine stops the auricle completely and inhibits the ventricle for as long as 8 minutes. Here the K picked up by the ventricle is less than that at 400 mg. KCl/l. and the cholinesterase activity is markedly depressed.

Effect of cortisone on pregnant ewes. LOUIS W. HOLM (introduced by S. ANDERSON PEOPLES), *School of Veterinary Medicine, Univ. of California, Davis.*

Previous studies in this laboratory have shown that nonpregnant ewes respond to injected cortisone with elevated blood glucose, transient plasma hyperchloremia and hypopotassemia. The blood cell picture is similar to that of other species (HOLM *et al. Am. J. Vet. Research.* 12: 314, 1951). Observations have been made on the effect of the hormone when administered during normal pregnancy and during a pathological state designated as lambing paralysis. Five animals were studied, all of which were within 2 weeks of term. Three ewes were normal; 2 were cases of clinically diagnosed lambing paralysis characterized by a blood glucose of 9–13 mg. %, and blood ketone bodies at a concentration of 32 mg.%. All animals were given 200 mg. of cortisone on the first day and 100–200 mg. each day thereafter for 4–10 days. Parturition among the normal animals was uneventful. One ewe with ketosis droped twin lambs on the 4th day of injections and died on the 5th day. The remaining animal dropped triplets on the 10th day of injections. Dam and lambs died on the 12th day. The ionic and blood cell changes observed in these animals were similar to those seen in nonpregnant ewes. In contrast to the data of Shaw *et al.* (*Science* 114: 574, 1951) relative to the effect of cortisone on postparturient hypoglycemia and ketosis in dairy cattle, there was no increase in the concentration of blood glucose from the preinjection level and no decrease in the concentrations of ketone bodies from the preinjection level. The responses of normal and ketotic pregnant ewes to cortisone more nearly correspond to those observed by Shaw in normal nonfasted lactating cows, than to those observed in bovine ketosis.

Histamine release in the human skin and the effect of cortisone. JAMES HOLMAN* AND ANDRES GOTH. *Dept. of Pharmacology, Southwestern Med. School of the Univ. of Texas, Dallas.*

The experimental drug RO2-2222 has been shown (*Federation Proc.* 10: 325, 1951) to liberate histamine in the dog and to cause typical histamine wheal and flare in humans, guinea pigs and dogs. This drug was used as a tool to study histamine release in the human skin and the effect of cortisone on this phenomenon. A solution of RO2-2222 in isotonic saline, containing 2 mg. of the drug/ml., was injected intradermally at varying intervals into human patients before and during cortisone treatment. The results of this study show that cortisone does not prevent the effect of a single injection of RO2-2222, but it makes the site of the injection less responsive to repeated injections of the histamine releasing agent.

Electroshock threshold and dose response to cortisone administration. DORSEY E. HOLTKAMP, LEO F. MANSOR AND ARTHUR E. HEMING (introduced by EDWIN J. FELLOWS). *Research and Development Div., Smith, Kline and French Labs., Philadelphia, Pa.*

Woodbury and Sayers (*Proc. Soc. Exper. Biol. & Med.* 75: 398, 1950) have demonstrated that cortisone administration produces a fall in electroshock threshold (EST) in rats. By following a similar technique with regard to the procedure and evaluation of the electro-shocking, we have investigated the relationship between the dose of cortisone acetate administered and the mean percentage decrease in EST. Five or 6 bilaterally adrenalectomized male rats (Albino Farms) of 200–250 gm. in weight, maintained on 1% saline as drinking water, were used for each dosage level. The cortisone (Cortone Acetate, Saline Suspension, Merck) was diluted to the respective dosage levels and administered to each rat in a single, daily, subcutaneous injection, using 0.2 cc. of 0.9% saline, 10% ethanol as the suspending vehicle. Four dosage levels were investigated: 0.025, 0.075, 0.25, 0.75 mg/rat/day. The respective mean percentage decreases in EST produced in the rats on the 10th day of drug administration were 4.6, 7.0, 9.4, and 11.6% when evaluated on the basis of pretreatment EST level. Plotting these mean percentage changes in EST against the log of the daily dose of cortisone gave a linear relationship.

Influence of alkyl substitution in the onium nitrogen centers of Mytolon. JAMES O. HOPPE AND A. ARNOLD.* *Pharmacology Section, Sterling-Winthrop Research Inst., Rensselaer, N. Y.*

Observations in the laboratory and the clinic have shown Mytolon (2,5-bis-(3-diethylaminopropylamino) - benzoquinone - bis - benzylchloride) to be a potent synthetic neuromuscular blocking agent. The molecule also possesses parasympathomimetic properties which are reflected particularly by sialorrhea in the clinic. Cholinergic manifestations are not conspicuous in the barbitalized dog. An enhanced vasodepressor response to both vagal stimulation and the injection of acetylcholine is demonstrable following subneuromuscular blocking dose levels of Mytolon in the barbitalized dog. Parasympathomimetic effects such as bradycardia and a copious water sialorrhea, however, are readily demonstrated in the unanesthetized

dog. These manifestations of parasympathetic stimulation appear to be associated with high anticholinesterase activity as determined by electrometric titration (approximately $\frac{1}{4}$ that of neostigmine methylsulfate). Changing the terminal alkyl groups from diethyl- to dimethylamino (WIN 3280) results in a reduction in anticholinesterase activity to approximately $\frac{1}{20}$ that of neostigmine which is associated with a reduction in parasympathetic stimulant properties and also in a reduction in curarimimetic activity in the rabbit and dog but not in the mouse.

Effect of EPN (ethyl p-nitrophenyl thionobenzenephosphonate) on human red blood cell cholinesterase. LEON HURWITZ AND VICTOR DISTEFANO (introduced by HAROLD C. HODGE). *Div. of Pharmacology and Toxicology, Univ. of Rochester, Rochester, N. Y.*

It was shown, by the use of manometric techniques, that EPN is a powerful inhibitor of human red blood cell cholinesterase. The compound behaves in a manner similar to that of many other alkyl phosphates: *1*) The inactivation of cholinesterase progresses with time. *2*) The addition of substrate (acetylcholine) arrests the progression of inhibition by EPN. *3*) There is no reversal of inhibition by substrate, since a 100-fold increase in acetyl-choline concentration did not alter the percentage inhibition of cholinesterase. *4*) EPN loses its inhibitory power on standing or by refluxing with .05 N NaOH for $\frac{1}{2}$ hour. Unlike the other alkyl phosphates which have only phosphorus to oxygen bonds in their molecular structures, EPN has a phosphorus linked to a carbon atom. Preliminary experiments carried out to characterize the increasing inactivation of cholinesterase with time indicate that the reation follows second order kinetics.

In vivo and in vitro effects of morphine on brain thiobarbituric acid (TBA) values. S. IRWIN* AND F. E. SHIDEMAN. *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.*

Zauder (*J. Pharmacol. & Exper. Therap.* 101: 40, 1951) reported that morphine, epinephrine, ACTH and cortisone, injected into rats chronically but not acutely, increased that ascorbic acid catalyzed oxidation of linolenic acid in the brain as measured by the thiobarbituric acid (TBA) test. He postulated that morphine, via the mechanism of epinephrine release (DE BODO *et al. J. Pharmacol. & Exper. Therap.* 61: 48, 1937), induced a release of ACTH from the hypophysis (LONG AND FRY *Proc. Soc. Biol. & Med.* 59: 67, 1945) with a consequent stimulation of the adrenal cortex and increased formation of linolenic acid. Forty-two chronic adrenal demedullated and 52 intact Wistar rats (200–280 gm.) were employed to test this hypothesis, as well as to check the possibility of morphine stimulation of the hypophysis directly or indirectly through a mechanism other than epinephrine release. Two groups of rats were employed, receiving daily subcutaneous injections of morphine sulfate (10 and 40 mg/kg., respectively) for a total of 42 days. At 7-day intervals, 20–22 hours postinjection, 3 or 4 adrenal demedullated and intact rats were sacrificed for study. In no instance was a significant increase in brain TBA values observed. This disparity in findings from those of Zauder may perhaps be due to differences in the time after injection at which the animals were sacrificed, a point which was not indicated in his paper. The matter, however, is presently under investigation in this laboratory and will be reported. Subcutaneous injection of a single dose of morphine (200 mg/kg.), with sacrifice of the animals at 1, 2, 4, 15 and 17 hourly intervals after injection, produced no increase in brain TBA values. However, direct addition of morphine (0.01–0.2% concentration) to the test system produced a reduction in the TBA values proportional to the concentration of morphine.

Experimental diabetogens: structure-response studies of styrylquinolinium compounds. HARRY K. IWAMOTO AND C. JELLEFF CARR. *Dept. of Pharmacology, Schools of Pharmacy and Medicine, Univ. of Buffalo, Buffalo, N. Y., and Dept. of Pharmacology, School of Medicine, Univ. of Maryland, Baltimore.*

Overshadowed by the prominence of alloxan as an experimental diabetogen since its first announcement in 1943 by Dunn, Sheehan and McLetchie, relatively little is known of the companion diabetogen described in the same report. The dye, 2-(*p*-acetylaminostyryl)-6-dimethylaminoquinoline methochloride, referred to by these workers as, 'Styrylquinoline No. 90,' produced rises of blood sugar levels reaching as high as 300 and 500 mg.% within an hour or so after intravenous administration to rabbits. Both the dye and its closely related derivatives have since been investigated, apparently without unanimity of results. The series by Lukens and Kennedy in 1949, notably their 'Compound VII.', provides the first semblance of confirmation. The purpose of present interest in the work briefly reviewed was to study the responses of certain of the pharmacodynamic structural minima suggested in the results of early investigators. The polemic nature prompted the syntheses of styrylquinolinium derivatives in accordance with the working hypothesis for requisite structure capable of diabetogenic activity. Pharmacodynamic studies in the rabbits and rats showed the compounds to be active in eliciting hyperglycemic end point with the typical fluctuating phases. The results indicated assurance of agreement with the trend seen in

the reported literature. It is felt that paucity of data of the literature regarding the source of the material, physical and chemical characteristics, variations of dosages, frequency of administration and postinjection blood levels, have contributed in a large measure to the difficulty in the recognition of unimpressive confirmation. Structure and diabetogenic response relationship is drawn.

Response of essential hypertension to semi-purified extracts of veratrum viride (Veriloid), using oral and intramuscular routes of administration. IRA JOHNSON AND LEWIS C. MILLS (introduced by R. A. HUGGINS). *Cardiac Clinic, Jefferson Davis Hosp., Houston, Tex.*

Although the original reports of Freis and coworkers (*Am. Heart J.* 36: 723; *Proc. Soc. Exper. Biol. & Med.* 72: 302) indicated that orally administered semi-purified extracts of veratrum viride (Vertavis, Veriloid) were effective in the treatment of essential hypertension, subsequent investigators (COE *et al. J.A.M.A.* 143: 5; MCNAIR *et al. Am. Heart J.* 40: 382) have had generally unsatisfactory results, and found regulation quite difficult at least on an outpatient basis. Twenty-eight patients have been treated with Anatensol, by mouth. The average maximum daily dose was 7.1 mg., but ranged from 3.2–12.8 mg. daily. A moderate response (decrease of 30 mm. or more systolic and 20 mm. diastolic) was obtained in 6 patients. In 4 others there was a questionable response. The remaining 18 failed to respond, even though dosage was increased to the point of incapacitating toxicity. In 10 private patients receiving Anatensol and 12 patients receiving Veriloid in the clinic, the results were similarly unsatisfactory. Despite the poor results obtained when these drugs were given orally, intravenous administration of Veriloid has produced prompt and consistent vasodepression, which persisted 1–4 days in another group of 17 patients. The mean systolic blood pressure for this group decreased from 243 to 156, and the mean diastolic pressure from 159 to 107. After the required dosage was established by intravenous administration, comparable results could be obtained by slightly larger doeses intramuscularly. A regimen was subsequently initiated, using combined intramuscular and oral therapy, in an attempt to reduce the frequency of intramuscular administration. The results of this method are to be presented.

A comparison of the serum cholinesterases of man and horse. WERNER KALOW. *Dept. of Pharmacology, Univ. of Toronto, Toronto, Ontario, Canada.*

Recognition and classification of esterases has made its greatest progress since artificial substrates were used. The necessity to use such substrates for investigation still exists, because there is not yet a general agreement as to the number and types of esterases occurring in the animal kingdom, and because their physiological substrates are not fully known. By using procaine as a substrate and as an inhibitor it has been possible to compare the properties of the serum cholinesterases of horse and man. When measured with u.-v. spectrophotometry, human serum hydrolysed procaine about 10 times more rapidly than horse serum. The Michaelis constants were 6×10^{-6} for procaine with horse serum esterase, and about 1.5×10^{-5} for the corresponding complex in horse serum. These figures were checked by using procaine as an inhibitor for the hydrolysis of acetylcholine in the Warburg apparatus. Procaine inhibited human serum esterase more strongly than horse esterase, and the degree of inhibition was as expected from the optically determined Michaelis constants. These results confirmed previous studies indicating that there is not a separate 'aromatic esterase' or 'procainesterase' in human serum to split procaine. They demonstrated, however, that cholinesterase in human serum differs in its properties from cholinesterase in horse serum.

Relationship between cholinesterase inhibition and ganglionic transmission in the cat. KAZUYA KAMIJO* AND GEORGE B. KOELLE. *Dept. of Pharmacology, Columbia Univ. College of Physicians and Surgeons, New York City.*

The effects of intravenous doses of DFP were measured in respect to *1*) inhibition of specific and nonspecific cholinesterase (ChE) activities of the pooled left superior cervical and stellate ganglia, *2*) height of isotonic contraction of the left nictitating membrane during maximal tetanic stimulation of the partially destroyed preganglionic trunk and *3*) antagonism of the relaxation of the membrane produced by injection of tetraethylammonium (TEA) into a branch of the common carotid artery during preganglionic stimulation. Cholinesterase activities of the ganglia were measured manometrically, using methacholine, butyrylcholine and acetylcholine as substrates. The right ganglia, removed prior to DFP injection, served as controls. A total of 47 cats was used; chloralose or pentobarbital was the anesthetic; most cats received 5.0 mg. atropine/kg. Following 0.25 mg. DFP/kg., which caused 50–60% inactivation of specific ChE and complete inactivation of nonspecific ChE, there was no definite increase in the height of contraction or antagonism of TEA; 0.5 mg., inactivating 51–83% of specific ChE, caused slight to moderate increase in contraction and antagonism; 1.0 mg., inactivating 87–89% of specific ChE, produced marked increase in contraction and antagonism. Doses above 2.0 mg., which completely inactivated specific ChE, caused

no further significant increase in contraction or antagonism; following cumulative total doses above 6–12 mg., depression of response occurred. Present results indicate that nonspecific ChE is not essential for normal ganglionic transmission, whereas inhibition of specific ChE beyond 50% produces increasing effects on transmission which are probably attributable to the enzymatic inhibition.

Correlation between pharmacological responses to benzoylcholine and acetylcholine and cholinesterase activity. ALEXANDER G. KARCZMAR.* GUY C. SHEATZ* AND THEODORE KOPPANYI. *Dept. of Pharmacology, Georgetown Univ. Med. School, Washington, D. C.*

It was suggested (KOPPANYI *et al.*) that the duration of action of benzoylcholine depends on circulating pseudocholinesterase. To test this suggestion *1*) activities of acetylcholinesterase and pseudocholinesterase were measured in blood and smooth muscle prior to and after administration of DFP and correlated with responses to benzoylcholine and acetylcholine (18 dogs). *2*) Benzoylcholine and acetylcholine responses were tested against injections of purified acetylcholinesterase; pseudocholinesterase and acetylcholinesterase activity was again measured in smooth muscle and blood (8 dogs). *1*) DFP in amounts capable of potentiating benzoylcholine but not acetylcholine response inactivated considerably or completely pseudocholinesterase but very little acetylcholinesterase of blood and smooth muscle; however, first observable potentiation of acetylcholine by more DFP was accompanied by considerable reduction of acetylcholinesterase in blood, smooth muscle and ganglia. Since pseudocholinesterase of blood and smooth muscle was inhibited to a similar degree, effects of DFP on muscarinic response to benzoylcholine (o.c.) may depend both on pseudocholinesterase in blood and effectors. *2*) Injected acetylcholinesterase did not diminish nicotinic (o.c.) and only slightly muscarinic effects of benzoylcholine while it inhibited completely pharmacological effects of acetylcholine. This agrees with contention that pseudocholinesterase but not acetylcholinesterase is concerned with termination of effects of benzoylcholine. It is noteworthy that although injected acetylcholinesterase was recovered from blood but not from sympathetic ganglia, preliminary results show some acetylcholinesterase penetration into smooth muscle, from which it can be recovered for longer periods than from blood. (Supported by the Council on Pharmacy and Chemistry of the A.M.A.)

Interaction of polarizing forces with inductive effects in the muscarinic moiety. HUGH H. KEASLING,* F. W. SCHUELER AND KENNETH LAMPE,* *Dept. of Pharmacology, College of Medicine, State Univ. of Iowa, Iowa City.*

In a previous report (Fall Meeting, The American Society for Pharmacology and Experimental Therapeutics, Omaha, Nebraska, October 15–17, 1951) we have noted the lack of muscarinic activity of ATB-1 and certain of its analogues. Subsequently we have prepared and tested for muscarinic activity the 5-methyl analogue of ATB-1. The apparent lack of muscarinic activity of these materials, which contain the appropriate atomic groupings and spatial relationships (i.e. interatomic O-N distances) for activity, is clarified by a consideration of the polarizing forces (cf., SCHUELER, F. W. *Federation Proc.* 11, 1952) of the groupings $(CH_3)_3N^+$ and $OOCH_3$ and their interaction. Indeed the utility of the calculation of polarizing forces as a tool in the clarification of moiety characteristics is emphasized by these materials. Although the discrete polarizing forces (P.F.) of the $(CH_3)_3N^+$ (P.F. = 3.21 dynes) and $OOCH_3$ (P.F. = −0.89 dynes) groups are the same as those for acetylcholine, the interaction of these groups by virtue of an inductive effect mediated through the conjugated system of the benzene ring diminishes the effective P.F. of $(CH_3)_3N^+$ in these materials to a much smaller value. The retention of activity in meta substituted derivatives, (e.g. Nu 2017/2 and other members of this series) follows by virtue of the lack of inductive interaction in such materials.

Effect of an autonomic blocking agent on morphine hyperglycemia. E. F. KEITH*, B. DEBOER AND R. K. H. FOSTER. *Dept. of Pharmacology, St. Louis Univ. School of Medicine, St. Louis, Mo.*

The effect of Lilly 08353 (1-phenyl-2N-methylbenzylaminoethyl-chloride HCl) upon the hyperglycemia subsequent to the administration of morphine was observed. For control purposes the effect of epinephrine by the subcutaneous and intravenous routes were studied. The effect of the blocking agent on this substance was then determined. In this series 2 groups of 6 animals each were used. There was no hyperglycemia observed in the intravenous group. The hyperglycemia produced by the subcutaneous administration of epinephrine was effectively blocked. In neither group did there appear a tendency toward hyperglycemia as a result of the administration of Lilly 08353. Twelve animals were then subjected to the effects of Lilly 08353, followed by a dose of morphine. In this group there was observed a significant hyperglycemic response ½ hour and 1 hour after administration of the blocking agent. Mor-

phine was then administered and the hyperglycemic response continued to be present to a significant degree ($P < 0.05$) throughout the whole period of observation. Three hours after the administration of morphine the peak concentration of blood sugar was observed. This was at the level of 121 mg. %, a concentration which compares well with the concentration of 124 mg. % observed in the ½-hour sample by DeBoer and Kimura for the peak level attained after the administration of morphine alone (personal communication). It is concluded that the blocking agent employed did not alter the degree of the morphine hyperglycemia, but delayed the onset of the peak effect as well as lengthening the duration of the hyperglycemic response.

Pharmacology of a 4-armed aliphatic methonium compound, tetrakis - (dimethylaminomethyl): methane tetramethobromide. C. J. KENSLER AND H. LANGEMANN.* *Dept. of Pharmacology, Cornell Univ. Med. College, New York City.*

In view of the striking pharmacological differences between pentamethonium and decamethonium, it was decided to investigate a series of 4-armed methonium compounds of varying chain lengths. The first (SKF 2091) of these compounds, with 3 carbons between each methonium head, has been synthesized by Dr. C. L. Zirkle of Smith, Kline and French Laboratories and has been found to be a highly active substance. In the cat, under Dial anesthesia, intravenous doses of 50–100 μg/kg. produced a marked transient rise in blood pressure, respiratory stimulation, and a contraction of the nictitating membrane. Larger doses (1–2 mg./kg.) produced respiratory failure and circulatory collapse. The pressor response to small doses was not blocked by atropine (1–2 mg/kg.), by TEA (10 mg/kg.) or by C_6 (0.4–0.6 mg/kg.). It was reversed by Dibenamine (15 mg/kg.). It was not influenced by adrenalectomy or by the use of a local anesthetic instead of Dial. The contraction of the nictitating membrane produced by doses of the order of 100 μg/kg was not blocked by sectioning preganglionic sympathetic trunk or by the excision of the superior cervical ganglion. The response was greatly reduced by atropine in concentrations which blocked the response to acetylcholine and markedly reduced the response to epinephrine. In these doses 2091 was without effect on the response of the membrane to continuous electrical stimulation of the preganglion nerve trunk. When TEA was used to depress the response to electrical stimulation by 50%, 2091 immediately restored the contraction to nearly maximal levels. The tone and motility of isolated cat duodenal strips was increased by 2091. This action was blocked by low concentrations of atropine but not by C_6. A contraction of the frog rectus was produced by 2091 and this action was blocked by D-tubocurarine. On the isolated rat uterus, 2091 was inactive in 400 times the concentration of acetylcholine which produced a contraction. However, the rat is much less susceptible to 2091 than the cat.

An evaluation of adrenal responses to ACTH and stress in the mouse. WOODROW B. KESSLER* AND JAMES LEATHEM. *Dept. of Physiology, Univ. of Texas Dental Branch, Galveston, and Bureau of Biological Research of Rutgers Univ., New Brunswick, N. J.*

A wealth of evidence has been accumulated to show that the adrenal glands of the rat react to ACTH and stress by depletion and their lipid content. In contrast, few of these data are available for the mouse. ACTH was subcutaneously administered to Swiss albino mice in 1-mg., 2-mg. and 3-mg. dosages and at each level animals were autopsied after 3, 6, 9, and 12 hours. Histochemical examination did not reveal any depletion in neutral fat and phospholipid, total neutral fat, unsaturated glycerides, aldehydes, or cholesterol. Similar results were obtained when ACTH was administered intraperitoneally or given in divided dosages (5 times/day for 10 days). Neither subjecting mice to cold stress for 12-hour periods, nor inducing 1–5 insulin hypoglycemic convulsions changed the fat histochemistry of the mouse adrenal from that of untreated control animals. Morphologic examination showed that the X-zone remained unchanged under ACTH administration, while slight hypertrophy of the fasciculata was noted in insulin stressed animals. Based on these findings, the following possibilities are suggested: *1*) The mouse adrenal has so great a supply of fat, that depletion is not noticeable histochemically. *2*) Different pathways exist for ACTH action in the mouse than in the rat and fat depletion might not be necessary for corticoid release. *3*) Repletion of fat in the mouse adrenal is remarkably rapid.

Interrelation of receptors for histamine and epinephrine in uterine smooth muscle. KAYE H. KILBURN, ABDULLAH A. ASADI, ALVIN C. MCCUISTION, RICHARD E. NILSSON AND EDWIN E. DANIEL (introduced by STEWARD C. HARVEY). *Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City.*

The effects of epinephrine, Dibenamine and histamine on diphenhydramine blockade of histamine and those of histamine, diphenhydramine and epinephrine on 688A (N-benzyl-N-phenoxyisopropyl-β-chloroethylamine) blockade of epinephrine were studied *in vitro* in the uteri of cats pretreated with progesterone. Dose-response curves for 2 uterine strips were determined simultaneously and were rapidly redetermined after 15-minute exposure to the blocking agent, 1 with and 1 without the agent being tested. All experi-

ments were run at 33°C. in Kreb's Ringer bicarbonate. Quantitative analysis of the results shows the following: at doses which are ineffective alone, diphenhydramine protects against 688A blockade of epinephrine and Dibenamine protects against diphenhydramine blockade of histamine. By our methods, histamine potentiates blockade of both epinephrine and histamine, but sometimes protects against a minimal blockade of both. Epinephrine, in contrast, protects against blockade of epinephrine, but potentiates blockade of histamine. Since Dibenamine and diphenhydramine have no direct actions on responsiveness, their interference with diphenhydramine and 688A, respectively, may indicate structural similarity, juxtaposition or partial identity of the receptor systems for histamine and epinephrine. Epinephrine protection against 688A blockade confirms other studies and indicates that 688A combines with the same receptor site as epinephrine. The ability of histamine and epinephrine (in the case of blockade of histamine) to potentiate blockade may be related to their inhibitory effects on responsiveness (see DANIEL, *Federation Proc.*, 11, 1952).

Further studies on hyperglycemia produced by morphine-like analgesic agents. K. K. KIMURA, E. F. KEITH* AND R. H. K. FOSTER. *Dept. of Pharmacology, St. Louis Univ. School of Medicine, St. Louis, Mo.*

The effect of codeine, dihydromorphinone, meperidine and Nisentil, (Roche) (DL-1,3-dimethyl-4-phenyl-4-propionoxy piperidine hydrochloride) on blood sugar was determined in fasted albino rabbits. The drugs were administered in the following i.v. doses (in mg/kg.): codeine 10, dihydromorphinone 1, meperidine 10, Nisentil 10. Venous blood samples were obtained before administration, and at ½, 1, 3, and 6 hours after injection of the drugs. Determination of blood sugar concentration was made on each sample by the photocolorimetric technique of Somogyi-Nelson. Tests were conducted at weekly intervals. The animals received the drugs according to a Latin square schedule. Control injections were made with physiological saline and paraldehyde 100 mg/kg. Hyperglycemic responses of significant degree ($P < 0.05$) were elicited in all animals, except the controls, at the ½, 1 and 3 hour observations. At the 6-hour observation the animals that received meperidine or Nisentil still showed a hyperglycemic level of significant degree when compared to the premedication level of blood sugar. The others had returned to a normoglycemic level by the 6th hour period. As in previous intravenous series (*Federation Proc.* 10: 314, 1951) with other analgesic compounds of a similar nature the peak response appeared at the 1 hour observation. In order of magnitude of hyperglycemia produced, the effective drugs were codeine > Nisentil > dihydromorphinone > meperidine. The percentage increases of blood sugar over the premedication level were in the same order as above: 107, 88, 63, 41, respectively.

Types and distributions of cholinesterases in the rabbit. GEORGE B. KOELLE. *Dept. of Pharmacology, Columbia Univ., College of Physicians and Surgeons, New York City.*

Ellis (*J.P.E.T.* 91: 370, 1947) has shown that the sera of all rabbits contain specific cholinesterase (ChE) whereas only some contain 'benzoylcholinesterase' (BzChE); moreover, all may contain a trace of 'pseudo-ChE' (LEVY, *J. Physiologie* 43: 127, 1951). The present study concerns the identification, characterization and comparison of the choline ester-hydrolyzing enzymes of rabbit tissues and sera. By fractional precipitation with sodium sulfate, the specific ChE and BzChE of rabbit sera were partially purified; similarly, a benzoylcholine-hydrolyzing enzyme was prepared from a homogenate of exsanguinated intestines. The substrate-pattern and susceptibility to inhibitors (eserine, DFP, Nu-1250, Nu-683) of the serum specific ChE was similar to that of the specific ChE of other species. The substrate patterns of the serum BzChE and the intestinal enzyme showed mutual resemblance; both hydrolyzed benzoylcholine at moderate, butyrylcholine at high, and acetylcholine at very low rates. However, the intestinal enzyme resembled the nonspecific ChE of other species in its susceptibility to the above inhibitors, whereas the serum BzChE was much more resistant to all excepting DFP. The effects of the inhibitors on butyrylcholine- and methacholine-hydrolysis by homogenates of whole tissues indicate that the intestine and superior cervical ganglion contain the same types of ChE's, whereas the liver contains additional enzymes which hydrolyze butyrylcholine. Histochemically, the distributions of specific and nonspecific ChE in the ileum and superior cervical ganglion resemble those in the cat, although a much higher proportion of ganglion cells in the rabbit contain specific ChE. Physiological implications will be discussed.

Effects of DFP on muscarinic responses of benzoylcholine. THEODORE KOPPANYI, GUY C. SHEATZ* AND ALEXANDER G. KARCZMAR.* *Dept. of Pharmacology, Georgetown Univ. Med. School, Washington, D. C.*

In nonatropinized dogs DFP (0.1–0.2 mg/kg.) enhances muscarinic effects of acetylcholine (0.0005–0.001 mg/kg.) on intestinal activity and mean arterial blood pressure, while it converts the muscarinic effects of benzoylcholine (0.09–0.15 mg/kg.) into nicotinic responses (rise of blood

pressure and intestinal relaxation). Atropine abolishes muscarinic effects of benzoylcholine; in most cases nicotinic effects are then obtained. Methacholine produces muscarinic effects, inhibited by atropine and not affected by DFP. In contradistinction to muscarinic the nicotinic thresholds for acetylcholine and benzoylcholine are comparable (0.1 and 0.25 mg/kg., respectively). The nicotinic responses are abolished by ganglionic depressants. Small doses of DFP potentiate 5–10 times nicotinic effects of benzoylcholine without affecting acetylcholine response. Increasing doses of DFP (up to 15 mg/kg.) never change responses to methacholine, inhibit or do not further potentiate benzoylcholine responses but, beginning with 0.5 mg/kg., potentiate progressively acetylcholine responses. It is concluded that among factors controlling response to cholinergic agents are *1*) affinity of effectors; and *2*) metabolic fate of these agents; benzoylcholine action is probably terminated in blood by pseudocholinesterase while that of acetylcholine depends also on true cholinesterase of blood and effectors. Methacholine is probably little affected by cholinesterases *in vivo* since its response is not changed by anticholinesterases. Additional postulates are necessary, however, as both methacholine and acetylcholine are destroyed 5–10 times faster than benzoylcholine (within 1 minute) on incubation in blood.

Influence of the hypophysis on copper metabolism. F. L. KOZELKA AND EDWARD PEDRERO, JR.* *Dept. of Pharmacology and Toxicology, Univ. of Wisconsin Med. School, Madison.*

Previous studies by the authors have demonstrated an *in vivo* relationship between copper and carbohydrate metabolism. Alloxan-diabetes and certain experimental hypoglycemias in the rat were found to produce a marked decrease of renal and hepatic copper levels. The present report deals with the effects of adrenalectomies, thyroidectomies and hypophysectomies on copper metabolism in the albino rat. Bilateral adrenalectomy or thyroidectomy had no effect on the copper levels in the kidney and liver. Extirpation of the hypophysis caused a marked decrease in the copper content in both the liver and the kidney. Excretion studies of copper in controls, in alloxan-diabetic and in hypophysectomized rats, revealed no significant differences in the fecal excretions in either the amount of feces or the copper content. There was, however, a very definite dissimilarity in the volume of urine and its copper content in the 3 series of rats. There was a very marked increase in urinary copper in both the diabetic and hypophysectomized animals. The results indicate that the hypophysis has an important influence on copper metabolism and that this metal is basically involved in the mechanism of carbohydrate metabolism.

Pharmacological and chemical relation between the veratrum alkaloids and the zygadenus alkaloids. O. KRAYER, B. H. ROGERS,* S. M. KUPCHAN,* AND C. V. DELIWALA.* *Dept. of Pharmacology, Harvard Med. School, and Dept. of Chemistry, Harvard Univ., Boston, Mass.*

Pharmacological studies with alkaloid mixtures from *Zygadenus venenosus* (YAFFE AND KUPCHAN, *Federation Proc.* 9: 326, 1950) confirmed the observation of Hunt (*Am. J. Physiol.* 6: xix, 1902) that the alkaloids have veratrinic properties, and showed that they possess circulatory and respiratory actions which are similar to those of some of the known veratrum ester alkaloids. Further pharmacological studies, along with the chemical separation of the alkaloid mixtures, indicated 2 types of alkaloids, 1 with properties similar to those of the ester alkaloids of *Schoenocaulon officinale*, GRAY, veratridine and cevadine, the other with properties similar to those of the polyacyl alkaloids of *Veratrum album*, L. and *Veratrum viride*, AITON, for example, protoveratrine or germitrine. The chemical fractionation and purification has now led to the identification of several crystalline ester alkaloids, 2 of which can be fully characterized, namely: veratroyl zygadenine, and vanilloyl zygadenine; a 3rd one is a germine ester, probably neogermitrine. The pharmacological properties of the zygadenine esters are very similar to the properties of veratridine on striated muscle, heart, circulatory reflexes and respiration. The germine ester belongs to the group of the polyacyl ester alkaloids which are characterized especially by the intense circulatory action (MAISON *et al. J. Pharmacol. & Exper. Therap.* 103: 74, 1951), which is relatively long lasting. Like protoveratrine, this substance lacks the typical veratrinic properties of the cevine and zygadenine esters.

In vitro oxidation of glycolic acid and glycolaldehyde by liver enzymes. ERNEST KUN. *Dept. of Medicine, Div. of Infectious Disease and Dept. of Biochemistry, Tulane Univ., New Orleans, La.*

Glycolic acid increases the O_2 uptake of liver slices. The magnitude of this increase in dependent on the rate of endogenous respiration. The oxidation of both glycolic acid and glycolaldehyde can readily be demonstrated when the endogenous respiration of liver slices is inhibited by azide, fluoride or iodacetate. The increase in O_2 uptake which occurs upon addition of glycolate is partially depressed if malonate is present. Glycolaldehyde alone inhibits the endogenous O_2 uptake of liver slices; malonate increases this inhibition. While the oxidation of glycolate is inhibited (80–100%) by hydroxylamine (5.10^{-3} M) in the presence of azide, glycolaldehyde oxidation is unaffected. Two different enzymes catalyze the oxidation of glycolic acid and that of glycolaldehyde. Pyocyanine is an effective electron carrier

for glycolic dehydrogenase, while methylene blue is not. The oxidation of glycolaldehyde, on the other hand, is increased by methylene blue. Glycolic dehydrogenase was purified by salt fractionation of rat liver extract. Upon prolonged dislysis the active fraction lost its ability to oxidize glycolic acid aerobically, unless pyocyanine was added to the test system. Properties of this enzyme, and the participation of glycolic acid and glycolaldehyde in cell metabolism will be discussed.

Bis quaternary aliphatic compounds. A. M. KUNKEL,* A. H. OIKEMUS* AND J. H. WILLS. *Pharmacology Branch, Chemical Corps Med. Labs., Army Chemical Center, Md.*

Twenty-two bis quaternary aliphatic compounds have been examined for potency in producing block of transmission through the superior cervical ganglion, in producing failure of vagal transmission to the heart, and as adjuncts to atropine in the prevention of poisoning by anticholinesterase agents. Maximal activity of these 3 sorts seems to be associated with a 5-carbon chain between the 2 quarternary heads, as shown in table 1. Replacement of the central carbon atom of the chain by S, N or O decreases activity in that order of increasing effect. The effect of variation in the structure of the quaternary head on activity is shown in table 2.

TABLE 1

Effect of Variation in Chain between Quaternary Heads

CHAIN	EFFECTIVENESS
$CH_2—CH_2—CH_2—CH_2$	43
$CH_2—CH{=}CH—CH_2$	43
$CH_2—CH_2—CH_2—CH_2—CH_2$	136
$CH_2—CH_2—O—CH_2—CH_2$	14
$CH_2—CH_2—S—CH_2—CH_2$	60
$CH_2—CH_2—N(CH_3)—CH_2—CH_2$	32
$CH_2—CH_2—CH_2—CH_2—CH_2—CH_2$	43
$CH_2—CH_2—CH_2—O—CH_2—CH_2—CH_2$	46
$CH_2—CH_2—CH_2—N(CH_3)—CH_2—CH_2—CH_2$	43

TABLE 2

Effect of Variation in Quaternary Heads

HEAD	EFFECTIVENESS
$N(CH_3)_3 \cdot Br$	53
$N(CH_3)_2C_2H_5 \cdot Br$	46
$N(CH_3)_2CH_2—CH—CH_2 \cdot Br$	60
$N(CH_3)_2CH_2—C(=O)—O—C_2H_5 \cdot Br$	60
$N(CH_3)_2CH_2—C_6H_5 \cdot Br$	1
$N(CH_3)_2C_{10}H_{21} \cdot Br$	1
$N(CH_3)_2C_{12}H_{25} \cdot Br$	60
$N(CH_3)_2CH_2CH_2—O—CH—(C_6H_5)_2 \cdot Br$	428 very toxic
$N(C_2H_5)_3 \cdot Br$	84

Use of the amenorrhea and menopausal index in the clinical evaluation of estrogenic compounds. HERBERT S. KUPPERMAN, MEYER BLATT,* HANS WIESBADER* AND SAKI TOGASHI.* *Depts. of Therapeutics and Obstetrics and Gynecology, New York Univ., College of Medicine, and Obstetrical and Gynecological Service (III Div.), Bellevue Hosp. New York City.*

In the past it has been very difficult to objectively and accurately evaluate the clinical efficacy of estrogens used in the treatment of the menopause and in priming patients with primary or secondary amenorrhea. To remove the possibility of subjective bias we have attempted to evaluate the comparative activity of estrogens by a statistical procedure through the use of mathematical conversion factors termed the menopausal and amenorrhea index. The menopausal index (M.I.) represents the sum of the numerical conversion of the severity of the 11 most common menopausal symptoms. Four of these; namely, vasomotor complaints, paresthesia, nervousness, and insomnia, have been given a weighted importance in formulating the menopausal index. The amenorrhea index (A.I.) represents the factor obtained by multiplying the extent of withdrawal bleeding noted after progesterone therapy by the quotient obtained by dividing the duration of bleeding by the time of onset of bleeding after a single intramuscular injection of 100 mg. of progesterone to a patient previously primed by 3 weeks of therapy with the estrogen to be evaluated. A new estrogen, tri-*p*-anisyl chloroethylene (TACE) has been evaluated by the above indices and has been compared with a large number of known estrogenic compounds. Our experiences with the use of the A.I. and M.I. in the clinical evaluation of some crystalline estrone sulfate compounds will also be presented. The use of the M.I. and A.I. in the clinical evaluation of estrogens administered by the 'blind testing technic' will be discussed.

Effect of age and adrenalectomy on the D-amino acid oxidase activity of rat liver. K. KURIAKI* AND C. J. KENSLER. *Dept. of Pharmacology, Cornell Univ. Med. College, New York City.*

The D-amino acid oxidase activity of the livers of newborn Wistar strain rats was found to be only

15% of the adult liver level. The activity increased rapidly and at weaning (*ca.* 21 days, weight *ca.* 30 gm.) adult levels were attained. The rate of increase is slower than that of succinoxidase but much faster than choline oxidase; 2 enzyme systems associated chiefly with the mitochondrial fraction of rat liver. Fractionation studies on D-amino acid oxidase in 18 rats disclosed that this enzyme is approximately equally distributed between the mitochondrial and supernatant fractions with less than 10% of the activity associated with the nuclear fraction. The distribution of this activity between mitochondrial and supernatant fractions varied markedly from animal to animal. In the same fractionation experiments, 70% of the activity of succinoxidase and choline oxidase was uniformly recovered in the mitochondrial fraction. In 7 adrenalectomized rats maintained on 0.9% saline, the D-amino acid oxidase activity was reduced by ¼ and examination of the activity of the cell fractions indicated that this loss occurred in the mitochondrial fraction. The supernatant fraction contained the same activity as this fraction from normal liver. The livers from both the normal and adrenalectomized rats contained 30.8 mg. nitrogen/gm. wet weight, and in the fractionation experiments 12% more nitrogen was recovered in the supernatant fraction of the adrenalectomized rats than in the same fraction from the controls. The riboflavin content of the livers of the adrenalectomized rats was not significantly different from that of the controls.

N-Phenacyl-N-(β-cyanoethyl)-piperidinium bromide: a material which potentiates the pressor response of epinephrine. KENNETH LAMPE* AND F. W. SCHUELER. *Dept. of Pharmacology, State Univ. of Iowa, Iowa City.*

N - Phenacyl - N - (β - cyanoethyl) - piperidinium bromide was originally synthesized during exploratory studies on the SAR of analgetic materials; this compound exhibited no analgetic properties at levels near the toxic dose by the Smith-D'Amour method. In the dog-carotid blood pressure preparation the compound, on slow injection, produced no change in blood pressure in relatively high doses (10 mg/kg.). Upon rapid injection there was a slight depressor effect, not blocked by atropine, and not effecting the pulse rate. Following the administration of this material (2–4 mg/kg.), the pressor response to epinephrine was markedly potentiated and the depressor phase was eliminated. This potentiation was effective immediately after injection and lasted, with gradual diminution, for several hours. Potentiation of epinephrine did not occur in the isolated heart preparation either with respect to amplitude or rate. The compound itself depressed the amplitude of the heart beat without concomitant change in heart rate or coronary flow. There was no significant change in the E.K.G. in rats even after administration of 4 times the toxic dose provided respiration was maintained. The depressant action on the amplitude of the heart beat is, therefore, probably a direct muscular effect. The LD_{50} for mice, by the method of probits, is 44 mg/kg. Death is apparently due to respiratory failure. Toxic doses produced epileptiform type convulsions in the mouse but the drug was not effective as an analeptic to pentobarbital. The convulsions could not be induced in frogs by injection into the ventral lymph sac. The SAR for epinephrine potentiation in this type of material will be discussed.

Methionine formation in liver homogenates. H. LANGEMANN* AND C. J. KENSLER. *Dept. of Pharmacology, Cornell Univ. Med. College, New York City.*

Conditions have been established for the measurement of methionine formation from homocysteine and choline, betaine or dimethylthetin in liver homogenates which yield higher Q methionine values than those previously reported by others. Evidence obtained using succinate to saturate proton and electron acceptors present in tissue homogenates supports Dubnoff's suggestion that in the rat choline must be oxidized before its methyl group is available for methionine synthesis. The use of succinate in the system increased the values obtained when betaine was the methyl donor but greatly reduced the values when choline was the methyl donor. The dimethylthetin-homocysteine transmethylase activity of mouse liver was twice that of rat liver. Rabbit, guinea pig and chick liver was less active than rat liver, chick liver activity being less than ⅓ that of rat liver. Betaine transmethylase activity did not parallel dimethylthetin transmethylase activity among the different species. Rat and mouse liver tumors showed little or no transmethylase activity. In chicks (obtained from Dr. Jukes and Dr. Burke of the Lederle Laboratories) deficient in folic acid or Vitamin B_{12}, transmethylase activity was not reduced. The folic acid deficient chicks yielded appreciably higher values than their supplemented controls.

Influence of certain inorganic ions on epinephrine action. EDWARD LARSON, MARK F. WYNN* AND JOSEPH P. ADAMS, JR.* *Dept. of Physiology and Pharmacology, Univ. of Miami Med. Research Unit, Veterans Administration Hosp., Coral Gables, Fla.*

In a previous report (LARSON AND ADAMS, *Federation Proc.* 9:294, 1950) compounds potentiating the blood pressure effects of epinephrine were studied, whereas in the present study, compounds having an opposite action will be considered. In-

organic salts of copper, iron, or nickel and others have produced a decreased pressor action when injected simultaneously with or previously to test doses of epinephrine into anesthetized animals. Small doses (2–4 mg/kg.) of ferrous, copper or nickel sulfate not only prevent the usual response to the simultaneous test dose of epinephrine but remain active in the body of the animal so that the expected response to a test dose is not produced for periods of 5–10 minutes. It is believed this inhibiting effect of these inorganic ions can be explained by their chelating properties.

Relation of dosage schedule to efficacy of terramycin. LOUIS LASAGNA AND C. G. ZUBROD (introduced by E. K. MARSHALL, JR.) *Dept. of Pharmacology and Experimental Therapeutics, Johns Hopkins School of Medicine, Baltimore, Md.*

A pneumococcal infection in mice was treated with parenteral terramycin hydrochloride. The duration of treatment was kept constant at 4 days. The effect of varying the period between injections from 4–48 hours was studied for each of 6 different total daily doses. With total daily doses of 25 mg/kg. or higher, essentially all the animals were saved, regardless of schedule. Daily doses of 3 mg/kg. had essentially no curative effect, the results again being independent of the spacing of doses. At 12.5 mg/kg., however, a clear-cut correlation between dosage schedule and efficacy was evident. The implications of these findings and the relation of the results to the height and duration of 'terramycin blood levels' will be discussed.

Failure to quantitatively account for infused inulin in human subjects. JULES H. LAST, RICHARD A. JONES,* GERALD O. MCDONALD* AND E. E. BOND.* *Dept. of Biochemistry, Northwestern Univ., and Research Service, Veterans Administration Hosp., Hines, Ill.*

It is generally accepted that inulin is quantitatively excreted in the urine within a 24-hour period in normal human subjects following parenteral administration. This concept is based on either single intravenous injections of variable doses of inulin or on continuous i.v. infusions over relatively short (6-hour) periods. During studies designed to yield mean 24-hour GFR values and inulin spaces in normal and edematous subjects, it was noted that a significant amount of infused inulin was not recovered in the urine. In 7 normal subjects, urines collected during the second 24-hour period following the end of an infusion yielded 'inulin' values comparable to pre-infusion urine blanks, yet a mean of 3.03 ± 0.65 gm. of inulin or 53.7 ± 6.51% of the infused amount remained unaccounted for. The amount of inulin 'lost' following 24 hours of continuous infusion seemed to be related to the duration of the infusion, since in 5 normal subjects infused for 6 hours, only 0.44 ± 0.35 gm. of inulin or 15.9 ± 1.2% of the amount infused could not be accounted for. In 4 patients with cardiac edema approximately 65.3 ± 10.9% of the infused inulin could not be accounted for after a 24-hour infusion. Available information rules appreciable loss in the bile. Significant loss in intestinal juices seems unlikely. The possibility of metabolism by the liver or other tissues must be considered. Such evidence would both preclude the use of inulin for the measurement of extracellular volume by the 'in-out' method and would render suspect inulin spaces based on the recovery of inulin in post-infusion urine.

Assay of purified extracts of veratrum viride based on the hypotensive effect in roosters. A. LAVALLEE, M. G. ALLMARK AND W. D. GRAHAM (introduced by F. C. LU). *Food and Drug Labs., Dept. of Natl. Health and Welfare, Ottawa, Ontario, Canada.*

An experimental design similar to Noel's procedure for epinephrine was used. (*J. Pharmacol. & Exper. Therap.* 84: 278, 1945). Roosters were prepared for the assay according to the procedure described in the U.S.P. XIV, page 476. The doses were injected into the alar vein at a constant rate of 0.1 cc/minute (in μg/kg. min.) for 10 minutes. Successive doses were given at intervals of not less than 45 minutes. The preparation of the extracts for assay and the criterion of responses were the same as described by Maison (*Arch. internat. de pharmacodyn. et de therap.* 85: 357, 1951). Roosters whose initial mean arterial blood pressure was above 90 mm. Hg were usually suitable for the assay. On the basis of preliminary trial 2 dilutions of the unknown were prepared to be about equal in potency to 2 dilutions of the standard and these were used for the assay. The range of blood pressure fall was between 25 and 50 mm. Hg. Several lots of tablets and powdered purified extracts have been assayed. In most cases 4-set assays (S_1, S_2, U_1, U_2 repeated 4 times) were done. Each preparation was assayed several times against the same provisional standard; the potency estimates tested for homogeneity, and a weighted mean and confidence limits computed. Within each series of assays the potency estimates were found not to differ and the standard errors, for $P = .05$, were less than ±20%. From a practical standpoint it would be preferable to use more than 1 rooster on each day or on different days, in each case to complete at least 2 sets (8 doses) on each assay. The desired precision is obtained by repeating the assay. Some of the potency estimates obtained by this method have been found to agree with those determined by the dog hypotensive method.

Effect of nicotine on the production of peptic ulcers in dogs. KWANG SOO LEE* AND CHARLES M. GRUBER, JR. *Dept. of Pharmacology, Jefferson Med. College, Philadelphia, Pa.*

It has been reported that 10–20% of the dogs receiving daily intramuscular injections of 30 mg. of histamine in beeswax for 5 days developed peptic ulcers. In this experiment histamine injections were combined with intramuscular injections of 16 mg. of nicotine daily. Histamine was prepared and injected according to the method of Hay, Varco, Code, and Wangensteen. Four mg. of nicotine was given 4 times a day at 2-hour intervals. All of the 9 dogs treated with histamine and nicotine developed peptic ulcers. The size, degree, and location of the peptic ulcers varied according to the animal.

A method for the production of disseminated necrosis and calcification in the cardiovascular system of albino rats. DAVID LEHR AND JACOB CHURG.* *New York Med. College, Flower and Fifth Avenue Hosps., New York City.*

The systematic production of mediocalcinosis in the arterial tree of albino rats has been described previously (Lehr, D. *Bull. New York M. Coll.* 6: 70, 1943; *Federation Proc.* 10: 318, 1951). The present paper deals with a greatly simplified and improved method which induces the desired alterations almost invariably and in a matter of a few days. The procedure consisted of a single intraperitoneal injection of sodium N^4-Acetyl Sulfathiazole in a dosage which produced severe 'obstructive nephropathy.' In addition to the medial necrosis of the arterial tree which followed the renal injury, most rats showed also cardiac injury, consisting of numerous foci of myocardial necrosis and perivascular infiltration of coronary arteries. The cardiovascular lesions were fully developed within 5–7 days following the injection of the noxious agent. Rats permitted to survive longer revealed correspondingly increasing degrees of reparative changes such as scar formation and intense calcification. Among 90 adult rats of both sexes subjected to the new procedure, the incidence of cardiovascular lesions ranged between 70 and 100% in different experiments. A further consistent finding was marked hypertrophy of the adrenals and atrophy of the thymus gland. In addition muscular necrosis was noted frequently in the stomach and at times also in the gut and the bladder as well as in voluntary muscle. As a rule hypertension appeared at about the 5th day after the injection and persisted for a minimum of 3–5 weeks. Peak systolic pressures of 180–300 mm. Hg were registered during this time. It is hoped that the simplicity and reliability of this short-term procedure will permit the evaluation of numerous factors considered of importance in the prevention and therapy of cardiovascular injury. (Supported by a research grant from the National Heart Institute, of the National Institutes of Health, U. S. Public Health Service.)

Antimuscarinic and antinicotinic actions of Toluidine Blue and Neutral Red in the intestine. JAMES L. LEITCH* AND THOMAS J. HALEY. *Atomic Energy Project, School of Medicine, Univ. of California at Los Angeles.*

The isolated intestine has been used to differentiate between the antimuscarinic and antinicotinic actions of Toluidine Blue and Neutral Red and comparison has been made with atropine. The intestine was stimulated with acetylcholine ($1:4 \times 10^7$), pilocarpine ($1:4 \times 10^5$) and nicotine ($1:2 \times 10^6$). With rabbit intestine, acetylcholine was completely blocked by atropine ($1:8 \times 10^5$) and Neutral Red ($1:2 \times 10^4$) whereas Toluidine Blue at its highest concentration ($1: 2 \times 10^4$) gave only 16% block. The muscarinic action of pilocarpine was completely blocked by atropine ($1:4 \times 10^6$) and Neutral Red ($1:1.67 \times 10^4$) whereas Toluidine Blue ($1:2 \times 10^4$) gave only 30% block. Nicotine was completely blocked by atropine ($1:5 \times 10^3$) and Neutral Red ($1:1.67 \times 10^4$), while Toluidine Blue was completely ineffective. Comparable data obtained with the guinea pig intestine will also be discussed. Both dyes are very poor antispasmodics compared to atropine but Neutral Red is more potent than Toluidine Blue. This may be related to the fact that the former compound has a nuclear nitrogen in the 9 position whereas the latter compound contains sulfur in this position. (Based on work performed under Contract No. AT-04-1-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.)

Mechanism of bile formation in the isolated perfused liver. Origin of the major inorganic constituents of bile. GEORGE F. LEONG,* R. W. BRAUER AND J. S. KREBS.* *Pharmacology Branch, Biological and Med. Sciences Div., U. S. Naval Radiological Defense Lab., San Francisco, Calif.*

In order to formulate the mode of action of the osmotic pump by which the hepatic cells excrete bile, it becomes necessary to examine the extent to which the inorganic constituents of bile participate in the pumping mechanism. Studies will be described concerning the time course of transfer of Na^{24}, Cl^{36}, $P^{32}O_4$, and several other labeled anions from the blood stream to the bile in the isolated perfused liver. The data obtained indicate that an equilibrium is rapidly established between plasma and bile with respect to Na^+ and Cl^-, while the more delayed course of bile $SO_4^=$ and $P^{32}O_4$ is in accord with the assumption of active transfer of these ions through the parenchymal cells. The applications of these studies to the formulation

of a working hypothesis regarding the mechanism of bile formation will be discussed.

Influence of thiamine deficiency on the accumulation of *p*-aminohippurate (PAH) by the rat kidney slice. DEAN A. LESHER* AND F. E. SHIDEMAN. *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.*

Albino, male rats weighing 70–120 gm. were placed on a thiamine deficient diet. Each rat was paired with another animal of approximately the same weight. This latter animal of each pair was either pair-fed or allowed to eat *ad libitum* a complete diet. On alternate days throughout the experimental period 24-hour urinary keto acid excretion (KNAUFF, R. E. Thesis, Univ. of Michigan, 1951) was determined on each animal. A deficient animal and his control were sacrificed 4–12 days after the appearance, in the deficient animal, of a peak in the excretion of urinary keto acids. The kidneys of both animals were removed, sliced and oxygen consumption and PAH accumulation by the slices determined by the method of Cross and Taggart (*Am. J. Physiol.* 161: 181, 1950). Renal slices from thiamine deficient animals were capable of concentrating PAH to a greater degree than were those from their pair-fed or *ad libitum*-fed controls. In general, kidney slices from deficient animals exhibited higher rates of oxygen consumption. Addition of sodium acetate (0.01 M, final concentration) to the medium in which the slices were suspended enhanced the uptake of PAH by both the deficient and nondeficient slices without producing any consistent or significant effect on oxygen uptake. In 2 experiments addition of diphosphothiamine (5 μg/ml.) to the medium suppressed PAH accumulation by the deficient slices and had no significant effect on concentration of PAH by slices from control animals.

Increase of post-tetanic facilitation in cat's soleus by ephedrine and yohimbin. TSUNG-HAN LI. *Dept. of Pharmacology, Harvard Med. School, Boston, Mass.*

Following the technique described previously (FENG AND LI. *Clin. J. Physiol.* 16: 1, 1941; LI AND TING. *Ibid.* 16: 37, 1941), epinephrine, ephedrine, and yohimbin were tested on the M. soleus of the cat under chloralose. Supramaximal single shocks were applied once every 10–60 seconds by a Grass stimulator delivering square waves with an intensity of 6 v. and a duration of 0.5 msec. Tetani of different frequencies were interposed. Normally the responses after high-frequency tetani, e.g. above 150/second were facilitated, but not after low-frequency, e.g. below 100/second. This post-tatanic facilitation was found to be significantly increased by ephedrine sulfate in doses from 0.5–2 mg/kg. given intravenously, intra-arterially, or by irrigation over the muscle. The Wedensky inhibition was always increased. Occasionally ephedrine sulfate caused some increase of twitch tension independent of tetanic stimulation. Epinephrine hydrochloride had no such effects. Yohimbin hydrochloride in the dose of 0.1 mg/kg. administered intravenously, or by irrigation, also produced some increase of post-tetanic facilitation, but not as strikingly as ephedrine. Contrary to ephedrine sulfate (0.5–2 mg/kg.) Yohimbin hydrochloride (0.1 mg/kg.) brought about very marked stimulation of the central nervous system and exhibited a strong neuro-muscular blocking action.

Physiological disposition of meperidine (Demerol) in man. PHILIP A. LIEF,* JOHN J. BURNS,* EMANUEL M. PAPPER, BETTY L. BERGER,* ALFRED WOLLACK AND BERNARD B. BRODIE. *New York Univ. Research Service, Goldwater Memorial Hosp. Dept. of Anesthesiology, Columbia Univ. New York City, and Natl. Insts. of Health, Bethesda, Md.*

A method has been devised which will assay as little as 0.3 μg. of meperidine/ml. of plasma. Using this method the physiological disposition of the drug in man has been studied. Less than 10 per cent of a dose of meperidine is excreted in urine. The drug is metabolized both through demethylation and de-esterification. By means of countercurrent distribution both the de-esterified (acid) and demethylated products of meperidine have been identified in urine. The rate of disappearance of meperidine from plasma varies from 10–20%/hour in different subjects. Repeated administration of the drug at 4-hour intervals does not result in appreciable accumulation. The rate of metabolism of meperidine in patients with terminal cancer who had acquired some tolerance to the drug is in the same range as in normal individuals. The dog and rat metabolize meperidine much more rapidly than man; 40 minutes after administration of the drug (20 mg/kg.) intravenously to the dog, over 80% of the drug had disappeared from the body. These results may explain the discrepancy observed between animals and man both as to the pharmacological effects and addiction liability. How differences in rates of metabolic transformation of drugs may explain quantitative differences in pharmacological activity and toxicity between man and animal will be discussed.

A comparative study of nonionic, cationic and anionic surface active agents on the frog heart. ALFRED G. LISI (introduced by CHARLES M. GRUBER). *Dept. of Pharmacology, Jefferson Med. College, Philadelphia, Pa.*

An assessment was made of the toxicity of 10 representative surface active agents on a single organ, the perfused frog ventricle. The nonionic

compounds were Tween 80, Triton X-100, Triton WR-1339 and Igepal CA Extra High Concentration; the cationic compounds were Zephiran Chloride and Hyamine 1622; the anionic compounds were Nacconol NRSF, Sodium 'Lorol' Sulfate PT, Sodium xylenesulfonate (Naxonate G) and Sodium benzenesulfonate. The lowest concentration which had a minimal inhibitory effect on contraction was determined for each compound. The first 8 compounds listed, with alkyl side chains of appropriate length to give them maximal efficiency in surface tension lowering, were much more toxic (0.0001–0.002%) than the last 2 compounds which lacked such side chains (0.25–0.87%). Within the group of the 8 more toxic agents, those which were least toxic showed the greatest depression of surface tension of the concentrations employed. There is a rough correlation between toxicity and high molecular weight with the exceptions of Trition WR-1339 and Tween 80. It would thus appear that the toxicity to the frog heart of the agents studied depends to some degree upon the size and complexity of the molecule. Hence the most toxic agents produced effects upon the heart without markedly altering the surface tension of the solutions employed.

Action of fluoride on the heart. O. Loewi. *Dept. of Pharmacology, New York Univ. College of Medicine, New York City.*

NaF (final concentration M/400–M/200) gradually increases the amplitude of the beats of the nonoptimally beating frogs heart (Straub preparation). The effect lasts almost indefinitely. NaF also restores the activity of hearts previously depressed by excess of K^+, by diminution of Ca^{++}, by Mg^{++} or citrate, and by long continued perfusion with Ringer. It was found that the restoring effect of fluoride in all these cases could also be obtained by applying oleate. The mode of action proposed here for both fluoride and oleate is based on the following considerations: it appears that Ca has to be partially fixed on the heart in order to fulfill best its well known function of preserving the normal physico-chemical state of the cells on which the ability to resist many kinds of injury depends. Though fluoride and oleate are quite different chemically, they share the properties of strongly combining with the heart and of forming sparingly soluble calcium salts. This would explain why these two agents both protect the heart against depressant influences.

A clinical study of prolonged anticoagulant effect with repository butacaine-heparin. Ted A. Loomis and J. E. Jesseph. *Dept. of Pharmacology, Univ. of Washington, Seattle.*

The relative insolubility of a previously described butacaine-heparin conjugate results in the slow liberation of heparin when the conjugate is injected intramuscularly. In the present study 50 adult hospitalized subjects were given the butacaine-heparin conjugate and a combination of the butacaine-heparin conjugate with free sodium heparin. It was found that 200 mg. of heparin as the butacaine-heparin conjugate injected intramuscularly would result in a 2-fold increase in the clotting time in 4–6 hours. The clotting time would remain at this level for the subsequent 8–10 hours and then slowly return to normal in 24 hours after the injection. Four hundred mg. of the butacaine-heparin conjugate would result in approximately a 3-fold increase in the clotting time. The clotting time remained at this level for the subsequent 12–18 hours and then slowly returned to normal at approximately 36 hours following the injection. The combination of 180 mg. of the butacaine-heparin conjugate with 180 mg. of sodium heparin, injected intramuscularly resulted in a 3- to 5-fold increase in clotting time within 1–1½ hours following the injection and the clotting time remained at this level for the subsequent 14–18 hours, slowly returning to normal 20–26 hours following the injection. No systemic toxicity was noted and the injections were painless. Two subjects developed grossly visible hematomas at the site of injection. Eight of the subjects had slight to moderate tenderness only on palpation at the site of injection on the day following the injection. This tenderness diminished over the subsequent 2–5 days.

Chemistry, pharmacology and oncolytic effect of the high molecular weight fraction isolated from podophyllin. Muriel R. Loran and Abe Towbin (introduced by Alfred Gilman). *College of Pharmacy and Dept. of Pathology, Ohio State Univ., Columbus.*

A high molecular weight fraction was isolated from podophyllin. The separation was accomplished by a process of dialysis and centrifugation. Podophyllin was dissolved in ethyl alcohol, and borate buffer solution was added to the alcoholic solution. The resulting suspension was dialyzed for 21 days at 1°C. The material remaining in the dialysis bags was then centrifuged to remove the insoluble material. The supernatant, representing the nondialyzable buffer-soluble fraction of podophyllin (NDBS) was a clear dark brown solution having a pH of 9.5. Electrophoretic analysis revealed the presence of at least 3 components; 2 of which were negatively charged (NDBSe2 and e3) and 1 which failed to migrate in the electric field (NDBSe1). Toxicity studies of the NDBS fraction and its components, NDBSe1, e2, e3, revealed the NDBS fraction to be toxic to white mice, causing anorexia, diarrhea, polyuria, decreased motor activity and death. Organs of animals autopsied during the 1st day showed micro-

scopically vascular lesions, with congestion and focal hemorrhages in the lung, spleen and intestine. The LD_{50} was determined at 84 mg/kg. The component responsible for the toxicity of the NDBS fraction is the NDBSe1 component with an LD_{50} at 7 mg/kg. NDBSe1 has an osmotic molecular weight of 23,000 and is believed to be a complex polysaccharide. The oncolytic effect of the NDBS fraction is to be presented at the Cancer Research meetings in April.

Assay of coronary dilator drugs in the isolated mammalian hearts and intact animals. F. C. Lu, M. G. Allmark,* Elizabeth J. Carmichael,* D. V. McMillan* and A. Lavallee.* *Food and Drug Labs., Dept. of Natl. Health and Welfare, Ottawa, Ontario, Canada.*

For the assay of Cortunon (a liver extract preparation, having coronary dilator activity) a method has been worked out and reported elsewhere (Lu, Canada. Physiol. Soc. Meeting, Kingston, Oct. 26, 1951). The assay was carried out on the isolated perfused rabbit's heart. Coronary inflow was recorded kymographically before and after injections on the preparations to be compared. In order to reduce the error caused by the changes in sensitivity of the coronary vessels, injections of the standard, one particular lot, and of the unknown were made alternately. The dose as well as the effect of the unknown were compared with the dose and the mean effect of the standard immediately preceding and immediately following the unknown. The potency relative to that of the standard and the standard error of the potency were then computed from those values according to the method described by Vos (*J. Am. Pharm. Assoc.* 32: 138, 1943). Significant differences in potency were found among the different lots of Cortunon assayed, the weakest having only about ⅓ the potency of the strongest. The method has also been found suitable for the evaluation of the coronary dilator activities of other drugs, such as aminophylline, Khellin, papaverine and Paveril. Adopting a similar design some preliminary experiments have been carried out with the aforementioned drugs on the isolated dog's heart perfused with defibrinated blood as well as the intact dog and appear to be satisfactory.

Mytolon and related compounds as antagonists of acetylcholine on the *Venus* heart. F. P. Luduena and T. G. Brown, Jr.* *Pharmacology Section, Sterling-Winthrop Research Inst., Rensselaer, N. Y.*

Mytolon (2,5-bis-(3-diethylaminopropylamino)-benzoquinone-bis-benzylchloride), a powerful neuromuscular blocking agent (Cavallito, Soria and Hoppe. *J. Am. Chem. Soc.* 72: 2661, 1950), and related benzoquinone derivatives were found to antagonize, *in vitro*, the inhibitory effect of acetylcholine (Ach) on the heart of *Venus mercenaria*. The concentration reducing by 50% the negative inotropic effect of 1:100 million Ach (EC_{50}) was estimated from the dose-response curves. The EC_{50} of Mytolon was 1:30 million. On a molar basis this compound is about 1000 times more active than tetraethylammonium. The other benzoquinone derivatives showed activity. Of those the benzyl chlorides were the most active. Dihydroerythroidine, decamethonium and *d*-tubocurarine had no activity. Sparteine showed only slight activity. Neostigmine and eserine were found inactive. It is concluded that there is no correlation between acetylcholine blocking activity on the *Venus* heart and ganglionic blocking, curarimimetic or anticholinesterase activity.

A new general anesthetic—isopropyl vinyl ether. Go Lu, C. Jelleff Carr, Mary Jane Fassel* and John C. Krantz, Jr. *Dept. of Pharmacology, School of Medicine, Univ. of Maryland, Baltimore.*

Isopropyl vinyl ether, isomeric with cyclopropyl ethyl ether ('Cypreth Ether'), is a volatile liquid eliciting anesthetic activity when administered by inhalation to rat, dog, monkey and man. The induction period is of short duration and the animals pass into deeper anesthetic planes with little struggle. There is little salivation and the abdominal relaxation is excellent. Pain reflexes are fully abolished and the recovery is rapid. It is approximately twice as potent as diethyl ether, retaining the same margin of safety. Under anesthesia neither the monkey's nor the dogs' hearts show significant electrocardiographic changes, and electroencephalograms in the dog and monkey are similar to those obtained with diethyl ether anesthesia. Blood chemistry study (urea nitrogen, sugar and carbon dioxide-combining power), bromsulfalein liver function test and clotting time determination in dogs reveal no significant difference before and after anesthesia. No hemolysis of dogs' blood is produced by 100 mg. % solution of the ether over a 24-hour period. No pathological changes of liver and kidney of rats and dogs can be observed under repeated anesthesias. In dogs, it is compatible with anesthesia induced with nitrous oxide or cyclopropane-oxygen mixtures and also with premedication with pentobarbital or morphine and atropine. The canine blood pressure is 10–15% lower under anesthesia with isopropyl vinyl ether than under diethyl ether. On perfused frog heart it is slightly more toxic than diethyl ether. This mixed ether compares very favorably with diethyl ether, divinyl ether and ethyl vinyl ether as an inhalation anesthetic.

Effects of plasma substitutes in unilateral traumatic shock in cats. L. M. Lusky* and H. A.

BRAUN. *Div. of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*

Cats were anesthetized with ether and tourniquets were applied to the right hind legs of the animals for 5 hours. (Because of the type of tourniquet used this resulted in a more severe test than that in experiments recently reported in *J. Antibiotics*, in press.) Comparable groups of these shocked animals were treated intravenously, 15 cc/kg., with solutions of Dextran 6%, polyvinylpyrolidone (P.V.P.) 3.5%, and dextrose 10%, all in isotonic saline. In 1 series treatment was initiated 30 minutes after release of the tourniquet. The animals were kept under observation for 72 hours and all deaths within this period were recorded. The number of animals surviving this period was selected as the most useful measure of the therapeutic efficacy of the plasma substitute administered. We were unable to demonstrate a significant difference in the therapeutic efficacy of Dextran, P.V.P., or dextrose using the above experimental design. In another series treatment was not initiated until 2 hours after release of the tourniquet. This experimental technique also failed to discriminate between the plasma substitute and dextrose. The average survival rate of these animals was 18%. When such animals were given an intramuscular dose of procaine penicillin G, 300,000 U/cat, 55 minutes after the start of the infusion of the intravenous solution, the average survival rate was increased to 49%. The difference is statistically significant ($P < .02$). Treatment with procaine penicillin G alone by intramuscular injection, had little effect in these severely shocked animals at a time when a large proportion of them were in the terminal stage.

Identification of elements of bone in pathological calcification. NORMAN S. MACDONALD AND M. M. ZACK (introduced by THOMAS J. HALEY). *School of Medicine, Univ. of California at Los Angeles.*

Studies have been made on several human ectopic calcifications occurring at areas of tendon insertion in the shoulder and fingers. Examinations by X-ray diffraction and histological methods showed that the viscous, white material aspirated from the area contained true bone salt and collagen. Attempts are being made to demonstrate the presence of muco-polysaccharide ground substance in the aspirated fluid. These findings are in accord with the modern viewpoint that pathologic calcification is not merely a precipitation of calcium and phosphate ions but also requires the preformation of an organic matrix in which true bone salt may then be deposited. (Based on work performed under Contract No. AT-04-1-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.)

Pharmacology of succinonitrile. DAVID I. MACHT. *Dept. of Pharmacology, Sinai Hosp., Baltimore, Md.*

Succinonitrile has been reported in Europe to be effective therapeutically in certain psychotic conditions and especially in depressive states. An intensive pharmacological and toxicological study of this compound was therefore undertaken by the writer. Toxicological experiments were made on mice, rats, guinea pigs, and rabbits and the MLD/50 for these animals was determined. The drug was found to be of very low toxicity. The effects of long-range administration of succinonitrile were studied on rabbits and were also found to be of low toxicity. Very little effect, if any, was produced on the blood picture. Kidney function was also unaffected and liver function studied by the bromosulfalein method of Rosenthal also revealed no harmful effects on the liver. The influence of the drug on the circulation and respiration was studied on the rabbit, the cat, and the dog. The toxicity on protoplasm was investigated by the author's phytopharmacological methods and found to be low. A most interesting finding was made in phytopharmacological studies of dilute solutions of the drug on blood sera of manic-depressive patients. It was found that succinonitrile detoxified the phytotoxic properties of this psychotic blood. In view of the low toxicity of the compound, clinical studies with it and pharmacologically controlled have been begun.

Urinary metabolites of C^{14} para-aminobenzoic acid. H. GEORGE MANDEL,* CHIANG-YING YEN* AND PAUL K. SMITH. *Dept. of Pharmacology, George Washington Univ. School of Medicine, Washington, D. C.*

The urinary metabolites of *p*-aminobenzoic acid in man have been reinvestigated with the C^{14} carboxyl labeled material. Using the Bratton-Marshall color test before and after hydrolysis, it has been shown that in normal man after an oral dose of 2 gm. the drug is excreted almost quantitatively within 8 hours. The metabolites were separated by counter-current distribution in a system of citrate buffer at pH 4.0 and isoamyl alcohol and by paper chromatography in saturated *n*-butanol. One half the compound was excreted as *p*-aminohippurate, ⅛ as acetyl *p*-aminobenzoate and ⅜ as approximately equal quantities of 2 substances that react as glycuronates, only 1 of which has a free amino group. Small amounts of free *p*-aminobenzoic acid and acetyl *p*-aminohippuric acid were also found. Ten microcurie doses of the radioactive compound were administered to each of 3 cancer patients to determine any additional metabolite of *p*-aminobenzoic acid.

It was found that the metabolites formed were identical, but that there was a great discrepancy in the ratio of the metabolites in normal and cancer patients. In the cancer patients the 2 glycuronates comprised almost all of the radioactive components of the urine. Only a small amount of acetyl *p*-aminobenzoate was found and only a trace of *p*-aminohippurate could be determined. There was no evidence of any other metabolites.

Urinary metabolites of C^{14} salicylamide. H. GEORGE MANDEL,* VICTOR W. RODWELL* AND PAUL K. SMITH. *Dept. of Pharmacology, George Washington Univ. School of Medicine, Washington, D. C.*

In an extension of the study of the metabolites of salicylic acid the urinary metabolites of salicylamide were investigated. Salicylamide was administered orally to normal individuals. The urine was hydrolyzed and analyzed by the Brodie-Udenfriend method for salicylates which showed that the metabolic products were completely excreted during the first 14 hours. Chromatographic analysis of the urine indicated the absence of free salicylamide or salicylic acid. Hydrolysis of the urine followed by chromatography showed the presence of salicylamide and indicated that the metabolites were conjugates of the drug. To aid in the identification of the metabolites, salicylamide-C^{14}-carboxamide was synthesized by ammonolysis of the methyl ester of tagged salicylic acid. Its purity was ascertained by absorption spectra, paper chromatography and countercurrent distribution. Doses of 300 mg. containing 2 mc. were administered orally to cancer patients, the urine collected for 16 hours, lyophilized and subjected to countercurrent distribution in a system of 0.1 M citrate buffer of pH 5 and isoamyl alcohol. The recovery of radioactivity in the urine was almost quantitative. Two major components were detected, and 1 has been identified as salicylamide by absorption spectra, distribution ratio, chromatography, and crystallization to constant specific actifity. The other component, a conjugate of salicylamide, apparently a glycuronate, accounted for more than ¾ of the activity.

Biological studies with radioactive morphine. C. H. MARCH* AND H. W. ELLIOTT. *Div. of Pharmacology and Experimental Therapeutics, Univ. of California, San Francisco.*

Morphine-N-methyl-C^{14} has been administered to rats to determine tissue distribution, metabolic fate and excretion. The labeled drug which had a specific activity of 3.75 μc/mg. was diluted 1:1 with ordinary morphine and injected subcutaneously in total doses of 5 mg/kg. Expired air and excreta were collected and tissues prepared for radioactivity analysis by the usual techniques. C^{14} was measured as barium carbonate. Male rats excrete 5–10% of the radioactivity via the respiratory route in 6 hours, indicating an active demethylation process, while females excrete less than 1% by this route. Both sexes eliminate virtually all radioactivity within 48 hours; about ⅔ via the urine and ⅓ via the gastrointestinal tract. Data from 1 animal with a cannulated bile duct indicate that the greater part of the radioactivity (61% in 6 hours) is excreted by the liver into the bile and is partially reabsorbed from the intestine and excreted by the kidneys in the normal animal. The central nervous system contained negligible amounts of radioactivity. Lungs and spleen contained significant amounts at 1 hour, but by 6 hours most of the radioactivity was in the urine or gastrointestinal tract. (Aided by a grant from the National Institutes of Health, Bethesda, Md.)

Curare compounds. XIV. Studies on the metabolism of carbon-14 labeled *d*-tubocurarine dimethylether in rats. DAVID F. MARSH. *Dept. of Pharmacology, West Virginia Univ., Morgantown, W. Va.*

d-Tubocurarine dimethylether iodide labeled with radioactive carbon atoms in 2 methoxyl groups and 2 nitrogen methyl groups was prepared. This material has an activity of 1400 counts/min/μg. Twelve male Carworth rats (200–240 gm.) were used in each of 2 experiments. Urine, feces, and expired air were collected separately for 24-hour periods. Intravenous injection of 30 μg. of the dimethylether/kg. (8,400–10,000 cpm) produces prompt head drop and partial skeletal muscle paralysis that lasts about 10 minutes; 25–40% of the total radioactivity is excreted in the urine, 0–4% in the air, and 0–6% in the feces in 24 hours; the amounts of material are too small for convenient handling. Intramuscular injection of 67 μg/kg. every 2 hours for 3 injections (2.8×10^5 cpm) produces some flaccidity but no true head drop. Four rats were decapitated after 3 hours and samples wet ashed and counted. The 24-hour urine samples of the remaining 8 contain 40–65% of the injected radioactivity, the feces 6–13, and the expired air 4–8%. The urine samples were pooled, adjusted to pH 8, extracted with chloroform, and lyophilized. The residue was extracted with ethanol and the ethanol extract precipitated with ether. Six % of the radioactivity is present in the tertiary base fraction (chloroform soluble), 22% soluble in absolute ethanol (later carrier studies indicate this is at least 65% unchanged dimethylether), 30% insoluble in ethanol, and the remainder lost during manipulation. The sacrificed rats have 40% of total activity in the urine fraction, 0.3% ml. plasma (2–3% total), none in blood cells, 4.0 in liver, 6.0 in kidney, 1.4 in brain, and 0.2/gm. wet muscle (about 20% in total muscle), or a total body recovery of about 75%.

Acute and sub-acute inhalation toxicity of hydrazine vapors to rats. FRANCIS P. MCGRATH,* CHARLES C. COMSTOCK* AND FRED W. OBERST. *Chemical Corps Med. Labs., Army Chemical Center, Md.*

The acute inhalation toxicity of hydrazine vapor was studied by exposure of groups of 6 rats for ½, 1, 2 and 4 hours to saturated vapors of hydrazine at 25°C. Skin irritation was noted and irregular periods of restlessness alternated with periods of inactivity. Profuse salivation occurred. The most severely affected animals showed marked increase of activity followed by convulsions terminating in death. Delayed deaths up to 14 days occurred among the less severely affected animals of each test. Gross examination of nasal passages revealed acute catarrhal rhinitis. A blood-like material present around the nares was identified as hemoporphyrin. Autopsy disclosed the presence of pulmonary edema in addition to destruction of the bronchiolar mucosa. Subacute studies were made by exposing groups of 20 rats to vapor concentration levels of either 300 mg/m.³, 76 mg/m.³ or 26 mg/m.³ for 6 hours/day, 5 days/week over periods as long as 6 weeks. During the exposure the animals appeared sleepy and were quiet. During the course of the experiment they lost weight. Deaths occurred in each of the 3 groups. The Lt50s were calculated as 27, 50 and 144 hours of exposures, respectively. Lungs, liver, spleen and kidneys showed pathologic changes as early as the 3rd day of exposure.

Ultraviolet absorption spectra and central nervous system activity of some 2-pyridyl compounds. DAVID MEGIRIAN* (introduced by IRWIN H. SLATER). *Divs. of Orthopedic Surgery and Pharmacology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Schueler *et al.* (*J. Pharmacol. & Exper. Therap.* 97: 266, 1949) reported that ultraviolet absorption spectra of some tertazole derivatives are related to pharmacological actions in the central nervous system: *1*) as absorbtivity height at 220 and 310 mμ decreased, stimulant action increased, *2*) as absorbtivity at 220 increased, depressant action increased. They indicated a general relationship may exist between spectra and pharmacological activity. A study of compounds related to α-phenyl γ-(2-pyridyl)-propyl ketone (EJA-II) did not confirm this hypothesis. The most potent depressant of the series shows the highest absorbtivity at 242 mμ, a peak attributed to the benzoyl moiety. Points of inflection are present at 261, 264, and 268 mμ corresponding to pyridine. Related ketones of somewhat lower potency show absorbtivity at 242–248 mμ, but potency does not correlate with the height of the peak nor do the height of the inflection points. (γ-(2-pyridyl) α-phenylpropyl methyl ketone) and (2-mesitylene-γ-(2-pyridyl)-propyl ketone) though similar in action to the above have no absorption peak at 245 mμ. Compounds with substitutions of a methyl, propyl, or phenyl group in the alpha position of EJA-II have similar spectra. The last, however is a convulsant and the others, depressants. The alcohols corresponding to the ketones exhibit the same pharmacological activity with slightly lowered potency, but have an absorbtivity $\frac{1}{20}$ that of the ketones at 268 mμ and no peak at 242–248 mμ. In this series ultraviolet absorption spectra do not correlate with pharmacological activity either qualitatively or quantitatively.

Enzymatic degradation of histidine and urocanic acid by liver and *Pseudomonas*. ALAN H. MEHLER* AND HERBERT TABOR. *Natl. Insts. of Health, Bethesda, Md.*

Previous work from this laboratory has established that degradation of histidine by extract of *Pseudomonas fluorescens* grown on histidine yields glutamic and formic acids and ammonia by way of urocanic acid. Isotope trapping experiments with unfractionated liver homogenates show that the initial reaction producing urocanic acid is the principal route of histidine degradation in this system, also. These results contradict the contention of Edlbacher that formation of urocanic acid by liver is of minor significance. No evidence was found for a reaction in which the imidazole ring of histidine is split prior to removal of the α-amino group. The enzyme histidase, which catalyzes the deamination of histidine to urocanic acid and ammonia, has been partially purified from both liver and *Pseudomonas*. This activity is easily measured by the appearance of the characteristic intense ultraviolet absorption of urocanic acid (extinction coefficient = 1.89×10^7 cm²/mole at 277 mμ). The disappearance of this absorption has also been used to measure the enzyme urocanase, which has been partially purified both from liver and from *Pseudomonas*. Studies on the mechanism of the over-all conversion of urocanic acid to L-glutamic and formic acids and ammonia will be reported. The α-N formyl derivatives of glumatic acid, glutamine, and isoglutamine have been synthesized, and their roles in the metabolism of urocanic acid will be discussed. An enzyme which rapidly hydrolyzes synthetic crystalline formyl glutamic acid to formic and glutamic acids has been partially purified from bacterial extracts. Some properties of this enzyme will be described.

Relation of 'chloramphenicol reductase' activity to chloramphenicol resistance in *Escherichia coli*. JOSEPH R. MERKEL AND EDWARD STEERS (introduced by C. JELLEFF CARR). *Dept. of Bac-*

teriology, Univ. of Maryland School of Medicine, Baltimore.

Previous studies on the enzymatic reduction of chloramphenicol by *Escherichia coli* questioned the effect of this activity on the primary mechanism of resistance. Since small inocula were always used, the possibility of small scale, cellwise reductions was not evident. Further experiments, utilizing the polarographic techhnique, were performed to demonstrate a relationship between chloramphenicol resistance and 'reductase' activity. Cells up to 10 hours old, harvested from brain-heart infusion media, had similar 'reductase' activities in growth media. Older cells showed a decrease in activity. 'Chloramphenicol reductase' activity in both growing and non-growing media varied directly with the chloro-cell contact time and with the cell mass. Experiments using nitrogen-free media demonstrated that high glucose concentrations inhibited the 'reductase' activity of the chloramphenicol-resistant cultures. Optimum 'reductase' activity of the resistant cultures was obtained with .002–.004M glucose. The chloramphenicol-sensitive cultures exhibited maximum activities between glucose concentrations of .002 and .016M. Similar pH optima (7–8) were found for both the resistant and sensitive strains. A study of the rate of chloramphenicol reduction by strains of *E. coli* having varying resistances to the drug indicated a relationship between 'chloramphenicol reductase' activity and the resistance exhibited. Further evidence for this relationship was found when the 'reductase' activity was compared with the loss of resistance by a highly resistant strain of *E. coli* subcultured daily in the absence of the drug.

Experimental myocardial infarction in the intact dog. FREDERICK H. MEYERS (introduced by ROBERT A. WOODBURY). *Div. of Pharmacology, Univ. of Tennessee, Memphis.*

The point of a spinal needle can be placed in the ventricular walls and septum of the hearts of lightly anesthetized dogs. Depending upon the orientation of the needle and the interspace through which entry is made, general areas can be located as desired and verified later at autopsy. After the injection of various caustics, of which 10 cc. of 4% mild protein silver has been most used, an area of necrosis is produced with slowly fibroses. The initial arrhythmia, unlike that following coronary artery ligation, appears irregularly, is transient and does not progress to ventricular fibrillation. The delayed ventricular tachycardia, appearing 8–24 hours later in most animals, is stable for a day or longer and is not converted to a sinus rhythm by procaine amide in doses (100 mg/kg i. v.) causing cardiac arrest. Interpretation of electrocardiograms and of the period of reduced cardiac output following the injection is complicated by a concomitant acute pericarditis, and weeks later the animals may develop a constrictive pericarditis. Preliminary work using agents which are primarily fibrosing rather than necrotizing, sodium morrhuate for example, suggests that the pericardial reaction can be avoided and that a preparation comparable to chronic congestive failure is possible.

Detoxication of nicotine by tissue slices. ARCHIE W. MILLER* AND P. S. LARSON. *Dept. of Pharmacology, Med. Collge of Virginia, Richmond.*

Tissue slices from dog, cat, rabbit and mouse have been investigated for their ability to detoxify nicotine when incubated with it *in vitro*. Detoxified nicotine was calculated from the difference between the amount of nicotine added and that recoverable unchanged, as determined by chemical means. Liver detoxified nicotine most rapidly in all 4 species. In order of decreasing detoxifying ability other tissues varied as follows: (dog) spleen, lung and skeletal muscle, aorta, kidney, intestine; (cat) intestine, kidney, spleen, lung and skeletal muscle, aorta; (rabbit) lung, intestine, kidney and aorta, skeletal muscle; (mouse) lung, kidney. Tissues studied that failed to detoxify nicotine were: (dog, cat) brain; (rabbit) brain and spleen; (mouse) brain, skeletal muscle and diaphragm. The only comparable studies have been reported by Werle and co-workers (*Biochem. Ztschr.* 298: 268, 1938; 308, 355, 1941; 313: 182, 1942; 318: 531, 1948) who in reporting on 3 of these species (dog, cat, rabbit) found detoxication of nicotine limited to liver, lung and kidney, kidney also being negative in the dog. Analysis of the above results indicate that the marked variation in the ability of the intact animal of these species to detoxify nicotine (*J. Pharmacol. & Exper. Therap.* 95: 506, 1949) cannot be satisfactorily explained by the rates at which their tissues detoxify the alkaloid *in vitro*. In further appears that *in vitro* detoxification of nicotine by tissue slices proceeds more rapidly than would be predicted from certain *in vivo* data (*J. Pharmacol & Exper. Therap.* 91: 357, 1947). (This study was made under a research grant from the American Tobacco Company.)

Traumatic shock in mice. Comparison of therapy administered in single and divided doses. R. CARL MILLICAN AND SANFORD M. ROSENTHAL. *Natl. Insts. of Health, Bethesda, Md.*

A standardized burn or tourniquet trauma was employed which was 95–100% fatal in untreated mice. Comparison was made of the survival response of the following fluids, administered in volumes of 5% of body weight: whole blood, plasma or serum, 5% serum albumin in 0.9% NaCl or in 5% glucose, and 0.9% NaCl alone. When therapy was administered in 1 dose no difference

in survival was obtained between whole blood, plasma, albumin in saline and saline alone. Albumin in glucose or glucose alone were much less effective. Under these conditions, therefore, the survival response was principally influenced by the sodium content of the fluid. When therapy was administered in 3 or 4 divided doses, given over a period of 7 hours following trauma, higher survival responses were obtained from the protein containing solutions than from saline alone. In the absence of sodium (albumin in 5% glucose) this effect was transitory and limited to the period of administration. Thus, in divided-dose therapy it was possible to demonstrate an effect from proteins, over and above that of the sodium contained in the solution. However, when the volume of saline administered at each injection was increased 50% above that of the serum, similar survivals were observed in both saline and serum treated animals. Saline was effective orally or intraperitoneally, as well as intravenously.

Effects of epinephrine on threshold electroshock after-discharge. BRUNO MINZ AND EDWARD DOMINO (introduced by KLAUS R. UNNA). *Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago.*

Recently Minz, Albe-Fessard and Buser have shown (*J. de Physiol.* In press) that epinephrine prolongs electrically induced seizures in rabbits. To analyze this phenomenon in spinal cats, sectioned at C1-C2, threshold electroshock convulsions were produced by condenser discharges (Goodwin stimulator) applied to the cortex by means of phonograph needle electrodes. The cortical after-discharge was recorded with an Offner EEG. Parameters of stimulation were varied in order to establish the experimental conditions under which threshold seizures were most reproducible. Frequencies of less than 10 cps were ineffective, while those from 20–1000 cycles produced convulsions of approximately the same duration. A frequency of 100 cps (pulse width 0.5 sigma) was selected for use in subsequent experiments. Intensities of 50–100 v. were the most effective when applied to the cortex for 1-5 seconds. The most consistent effects were obtained by using a range of increasing time intervals from 5 seconds–30 minutes between electroshocks. When the duration of after-discharge was plotted against ascending time intervals, a reproducible curve was obtained. This consisted of a response to the initial shock, then, to stimuli delayed approximately 5–30 seconds, there was a refractory period during which no after-discharge was obtained. With further increase in intervals between stimuli, seizure responses were again obtained and were frequently more prolonged than the initial shock. Synthetic epinephrine or nor-epinephrine given intravenously or intra-arterially in doses of 1–10 μg/kg., modified this curve by prolonging cortical after-discharge, and shortening the refractory period to subsequent shocks. These effect soccurred from 30 seconds to several minutes following injection and were independent of the blood pressure response. Glucose administered intravenously did not affect the electroshock seizure.

Determination of the sympathetic mediator in various species. BERNARD L. MIRKIN* AND DESMOND D. BONNYCASTLE. *Dept. of Pharmacology and Toxicology, Yale Univ. School of Medicine, New Haven, Conn.*

Differences in the nature of the neuro-humoral agents released in different species upon sympathetic nerve stimulation have been described by West (1949). An investigation has been made to determine the ratio of nor-adrenaline(N-A) to adrenaline(A) which occurs in perfusates obtained from different organs in the body, both before and after stimulation of sympathetic fibers present in these areas. The method of analysis employed is a paper chromatographic separation of the amines, followed by elution and a quantitative determination performed utilizing the rat ascending colon (N-A) and rat uterus (A). Concentrations of 2.5×10^{-8} nor-adrenaline and 5×10^{-9} adrenaline can be ascertained in this fashion. The present work will discuss: *1*) The relationship between the N-A/A ratio found in the adrenal medulla and that observed in the different perfusates to determine whether the ratio in the medulla indicates the nature of the mediator throughout the entire body; *2*) The differences between the over-all N-A/A ratio and its significance in the response (hemodynamic and metabolic) of these species to parenteral injections of nor-adrenaline and adrenaline.

Method for determination of quarternary ammonium compounds in biological media. RUTH MITCHELL* AND BYRON B. CLARK. *Pharmacology Dept., Tufts College Med. School, Boston, Mass.*

The increasing pharmacological interest in quarternary ammonium compounds stimulated our efforts to develop a method for their determination in biological media. The problem in finding a suitable method revolved around the difficulty of quantitatively extracting the highly ionized quarternary salts from biological media without contamination by metabolites or naturally occurring organic bases. The method reported here is a modification of Auerbach's method (*Anal. Chem.* 15: 492, 1943) adapted for use with biological material and capable of measuring quantities as low as 2.5 μg/sample. The method is based on the reaction of the anion of bromphenol blue (0.04% in

1M K_2HPO_4), and the cation of a quarternary ammonium compound to form a stable colored dye complex in alkaline solution. This reaction can be carried out in the original mixture and then the colored complex can be quantitatively extracted with ethylene dichloride without interference from the unreacted dye. The presence of phosphate-ion was found essential for full color development. At present, this complex salt formation seems to be a property only of quarternary ammonium bases in which R_1, R_2 and R_3 can be CH_3 or longer chains, and R_4 must be C_4 or longer or $C_6H_5CH_2$ or modifications thereof. A large number of quarternary ammonium compounds of diverse chemical structure have been determined by this method. All tertiary amines tested have given negative results. In certain cases where the protein content of the original media was high, the method has been modified to insure complete recovery of the quarternary ammonium salt.

Vasodilator and adrenergic blocking action of 6-allyl-6,7-dihydro-5-dibenz [c, e] azepine phosphate (Ro 2-3248). PAUL E. MOORE,* ALFRED W. RICHARDSON* AND HAROLD D. GREEN. *Dept. of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.*

Blood flow in the femoral artery of the dog, anesthetized with Na pentobarbital, was measured with the electrically operated direct writing differential pressure flowmeter of Green, Richardson and Denison (*J. Lab. & Clin. Med.* In press), and arterial pressure just distal to the flowmeter was recorded by a Statham pressure gauge. Intra-arterial injection of 1 μg. of epinephrine caused vasoconstriction (increasing the peripheral resistance, PRU, to 160–880% of control). Approximately 1 mg/kg. (6–12 mg.) of Ro 2-3248 injected intra-arterially was required to block completely the epinephrine induced constriction. 1 μg. of epinephrine then caused marked vasodilation (PRU = 30–36% of control). After this dose of Ro 2-3248 up to 12 μg. of epinephrine induced only vasodilation and up to 60 μg. of epinephrine intra-arterially caused vasodilation after an initial phase of vasoconstriction. The intensity and duration of the phase of vasodilation increased with the dose of epinephrine. Absence of constriction in response to 1 μg. of epinephrine intra-arterially persisted for 1.5-6 hours, and the phase of dilation persisted for 1.2–5.5 hours after 1 mg/kg. of Ro 2-3248. Smaller doses of Ro 2-3248 converted the response to 1 μg. of epinephrine to a short phase of partial constriction followed by a phase of dilation. Intra-arterial injections of Ro 2-3248 alone caused temporary vasodilation (PRU = 80–32% of control for 0.5 mg., 60–17% of control for 0.5 mg/kg. and 10–7% of control for 1.0 mg/kg.). The vasodilation decreased only slightly or not at all with repeated injections of Ro 2-3248. (This work was supported by grant H-487(C2) from the National Heart Institute.)

Effect of DL-amphetamine (Benzedrine sulfate, S. K. F. Labs.) on voluntary choice of ethanol by albino rats. WILLIAM T. MOORE,* BERNICE M. MOORE,* JOE B. NASH* AND G. A. EMERSON. *Univ. of Texas Med. Branch, Galveston.*

Seven of 18 rats, treated subcutaneously with 4.0 mg/kg/day of amphetamine sulfate for 2 nonconsecutive 7-day periods during different times within 35 days, and 0.9% NaCl subcutaneously for 14 days during the remainder of this period, showed a decreased intake of 5% V/V alcohol solution during both courses of amphetamine. An additional 9 rats showed a decrease during either the 1st or 2d course of amphetamine. Decreases were most marked in rats with an initially low preference for alcohol. Difficulties in extrapolating these results to man are noted.

Observations of cardiac rhythm during ethyl chloride anesthesia in the dog. LUCIEN E. MORRIS, BRUNO HAID AND JOSEPH M. WHITE, JR. (introduced by STUART C. CULLEN). *Div. of Anesthesiology, State Univ. of Iowa College of Medicine, Iowa City.*

Electrocardiographic observations were made on dogs during both induction and maintenance of anesthesia with ethyl chloride. Cardiac irregularities of nearly all types occurred including ventricular stand-still which followed either relative or frank overdosage. Sensatization of the myocardium by ethyl chloride to subsequent administration of epinephrine was shown. Test injections of epinephrine according to a standard technic (MEEK, HATHAWAY AND ORTH. *J. Pharmacol. & Exper. Therap.* 61: 240, 1937) usually produced ventricular tachycardia. In some instances, however, marked slowing, blocks, and asystole were also observed.

Effect of diphenylhydantoin sodium (Dilantin) on digitalis arrhythmias. LOS MOSEY, MYRA D. TYLER AND ROBERT O. BAUER (introduced by GEORGE L. MAISON). *Dept. of Pharmacology, Boston Univ. School of Medicine, Boston, and Cushing Veterans Hosp., Framingham, Mass.*

The effectiveness of Dilantin abolishing ectopic ventricular activity in dogs with acute myocardial infarction was demonstrated in 1950 by Harris. The present study was undertaken to determine whether similar arrhythmias produced by excessive doses of ouabain could be alleviated by Dilantin. To 10 adult mongrel dogs ouabain, 0.07–0.09 mg/kg., was administered intravenously. Ventricular arrhythmias, ranging from numerous multifocal ectopic beats to ventricular tachycardia

were produced in all dogs. (Such arrhythmias have been found to persist more than 6 hours if no treatment is employed.) Dilantin 5 mg/kg/min. (total dose of 10–30 mg/kg.) was then infused. Electrocardiograms were taken before ouabain, after arrhythmias had developed, during the Dilantin infusion and at 5-minute intervals thereafter as indicated. Sinus rhythm was reestablished during the infusion of Dilantin in every instance. In 7 of the animals ventricular arrhythmias recurred. Duration of response to Dilantin (30 minutes–3 hours) was demonstrated by return of ventricular arrhythmias. Nystagmus and tremor were observed in 2 animals following administration of Dilantin. Dilantin 0.6 gm/day in divided doses was administered to 4 patients manifesting ventricular ectopic activity as a result either of digitalis toxicity or cardiac disease. In every patient ectopic activity was reduced or disappeared. When subjective symptoms of the ectopic beats had been noted, those were alleviated.

Clinical diuretic studies on 3 new mercurial compounds. JOHN H. MOYER AND CARROLL A. HANDLEY. *Depts. of Medicine and Pharmacology, Baylor Univ. College of Medicine, Houston, Tex.*

Three new compounds, 3-chloromercuri-2-methoxypropylurea (1347Ex), 3-carboxymethylmercaptomercuri-2-methoxypropylurea (1353Ex), and 3 - (α - carboxyethylmercaptomercuri) - 2 - methoxypropylurea (1431Ex), have been found to have 3–4 times the diuretic potency of meralluride when administered to dogs and the drugs are compared according to mercury content. Therefore, clinical evaluation was undertaken on 107 moderately severe to severe cardiac patients, using dosages equivalent to 10, 20, and 40 mg. Hg intramuscularly twice a week for 2–3 months. All of these patients were previously maintained on Mercuhydrin equivalent to 80 mg. Hg content twice a week. Sixty-one received 1353Ex, 24 received 1431Ex and 22 received 1347Ex. The only untoward reactions were diarrhea in 1 patient receiving 1353Ex and pain on injection in 2 receiving 1431Ex and 1 receiving 1347Ex. Clinically, the 3 drugs appeared to be about equally effective. Dosages equivalent to 10 mg. Hg i.m. were too small and about 25% of the patients developed failure. However, on dosages equivalent to 40 mg. Hg all patients were well maintained. An occasional patient who was maintained on 40 mg. would develop failure if only receiving 20 mg. Electrolyte studies carried out on 53 additional patients revealed that all of the new compounds were superior to Mercuhydrin in promoting sodium and water excretion. Of the newer compounds 1347Ex appeared to be superior in this respect.

Oxygen uptake and ATPase activity of the heart and arterial tissue. RUTH MUSSER,* JOHNSON LING* AND JOHN C. KRANTZ, JR. *Dept. of Pharmacology, School of Medicine, Univ. of Maryland, Baltimore.*

The oxygen uptake and ATPase activity of heart muscle and aorta have been studied in the rat with respect to the vasodilators of the nitrate and nitrite series. It was shown that the oxygen uptake of rats' aorta was depressed by sodium nitrite in therapeutic concentrations but that the oxygen uptake of the heart was not affected by the same concentration of sodium nitrite. Glyceryl trinitrate affected both oxygen uptake and ATPase of rats' aorta in therapeutic concentrations. Neither of these 2 facets of the energy-producing system of the heart was affected by the glyceryl trinitrate. These experiments indicate that vasodilators of this series exhibit specificity for the energy-producing systems of the blood vessels.

Drug effects on the metabolism of *Endamoeba histolytica*. MITSURU NAKAMURA* AND HAMILTON H. ANDERSON. *Div. of Pharmacology and Experimental Therapeutics, Univ. of California, San Francisco.*

Cultures of *E. histolytica* in association with a 'streptobacillus' grown in liver-proteose-peptone medium were utilized to study the inhibitory effects of representative amebacides. Penicillin was added to inhibit the associated bacterium prior to experiment and the cultures were sea'ed to prevent oxidation. Amebas from several cultures were pooled, washed and centrifuged. Approximately 1 million amebas were placed in ea h Warburg vessel with 1 ml. of 0.01 M sodium bicarbonate, 10 mg. penicillin G, 5 mg. streptomycin, 5 mg. cysteine, and 10 mg. glucose. Flasks were cleared of air by use of 5% CO_2 plus 95% N. During experiment, measurements of gas production, as acid or CO_2, were made every 10 minutes over 70 minutes. Test drugs were added 60 minutes beforehand. Emetine hydrochloride, 1/20,000 dilution, caused 50% inhibition of total metabolism of the ameba. Fumagillin, at 1/20,000, caused complete inhibition of metabolic activity. Terramycin (generously supplied by the producers), at 1/20,000, did not inhibit, and at 1/5,000 caused only slight inhibition. Control cultures of the associated bacterium, exposed to test drugs, and of the ameba untreated, were made simultaneously. The method, to be reported in detail elsewhere, provides a more precise technic for study of the metabolism of *E. histolytica* than others now available. (Supported in part by the National Institutes of Health, Bethesda, Md.)

Hemodynamic effects of lidocaine solutions. MARK NAKAUCHI AND ALEXANDER G. KARCZMAR (introduced by EDWARD B. TUOHY). *Depts. of Oral Surgery and Pharmacology, Georgetown Univ. Med. and Dental School, Washington, D. C.*

These experiments were carried out in dogs anesthetised with Nembutal or paraldehyde. Two problems were studied: *1*) whether lidocaine when compared with cocaine and procaine potentiates the pressor effects of epinephrine; *2*) what are the amounts and concentrations of epinephrine in lidocaine and procaine solutions sufficient to produce pressor effects upon intravenous and oral submucosal injection. Cocaine consistently, procaine in 60% of cases and lidocaine (up to 10 mg/kg., i.v.) does not potentiate pressor effects of epinephrine. Intravenous administration of procaine and lidocaine alone produces only vasodepressor responses. Intravenous injections of epinephrine-containing procaine or lidocaine (2%) solutions may cause rise in mean arterial blood pressure, minimal effective dose (MED), 0.03 cc. with 1:100,000 epinephrine in both cases, producing 3.1 mm. Hg rise. Even 1 cc. with 1:50,000 epinephrine produced only a 16-mm. Hg rise. In case of submucosal injections in 11 oral regions most pronounced pressor effects were obtained from anterior palatine region, 0.25 cc. of 2% lidocaine with 1:100,000 epinephrine (MED) causing a 3-mm. Hg rise; 2 cc. with 1:50,000 epinephrine caused a 16-mm. Hg rise. Massage caused pressor effects only in posterior palatine region (1 hour after injection). It is concluded that *1*) lidocaine has little or no sympathomimetic properties; *2*) pressor effects of procaine and lidocaine solutions containing epinephrine are caused by, and are proportional to, their epinephrine content; *3*) lidocaine solutions containing epinephrine are not likely to produce pressor effects in amounts used in dental practice.

Effect of anosmia on voluntary choice of dilute ethanol by albino rats. JOE B. NASH,* WILLIAM T. MOORE,* BERNICE M. MOORE* AND G. A. EMERSON. *Univ. of Texas Med. Branch, Galveston.*

Technics of quantifying preference for alcohol in rats (RICHTER AND CAMPBELL. *Science* 91: 507, 1940; MARDONES *et al. Arch. Biochem.* 9: 401, 1946; WILLIAMS *et al. Texas Repts. Biol. & Med.* 8: 238, 1950) are based, at least in part, on normal functioning of the chemical senses. Richter (*loc. cit.*) notes 'taste thresholds,' and further that study is in progress on effects of ablation of the olfactory lobes on these 'overlong periods of time'; while maximum life expectancy of rats may be a maximum of 4 years, we have thus far noted no reports of such studies. Ablation of the olfactory lobes of the c.n.s. in rats causes decreased intake of alcohol in rats with initially high preference for alcohol, and an increased intake in those with a low preference; but in neither case do the postoperative preferences approach the theoretical 50%. Similar results follow repeated irrigation of the nostrils with 2% $ZnSO_4$. If K tellurite is repeatedly given i.m. in doses causing marked respiratory excretion of dimethyl telluride, which might fatigue olfactory endings, rats with both high and low preference for alcohol greatly decrease their alcohol intake. Subsequently, oral treatment with methionine or betaine bases causes a further decrease during the recovery period of increased preference for alcohol.

Effect of liver on blood levels of 17-hydroxycorticosteroids in the dog. DON H. NELSON AND BOYD HARDING (introduced by LEO T. SAMUELS). *Dept. of Biochemistry. Univ. of Utah College of Medicine, Salt Lake City.*

Previous work has demonstrated the rapid disappearance from the blood stream of free cortisone injected intravenously. This study was initiated to determine what tissues were chiefly responsible for this removal. The levels of 17-hydroxycorticoids in the blood were determined by the method of Nelson and Samuels. The injection of 5–25 mg. of free cortisone intravenously into dogs weighing 9–18 kg. gave maximum blood levels of 50–200 γ 17-hydroxycorticosteroids/100 ml. blood. These levels fell rapidly after completion of the injection, and were almost back to normal within 60 minutes. Arterial and hepatic venous blood samples drawn simultaneously showed marked differences across this organ. There was no significant difference between simultaneous arterial and renal venous values or between other arterial and venous samples taken across other tissues. It appears that the liver plays a major role in the metabolism of the adrenal steroids.

Effect of an adrenergic blocking compound (Regitine) on the course of alloxan diabetes. JOHN NICHOLS* AND J. MAXWELL LITTLE. *Dept. of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.*

Following the report of Trapold *et al.* (*J. Pharmacol. & Exper. Therap.* 100: 119, 1950) that oral or intravenous administration of the adrenergic blocking compound (Regitine) to dogs resulted in hypoglycemia and prevented the hyprglycemic response to injected epinephrine, the effect of this drug on the course of alloxan diabetes was studied. The oral or intravenous administration of Regitine to dogs, in doses in excess of those used by Trapold *et al.*, resulted in a slight decrease in blood sugar concentration with no observable symptoms. The dogs were then given alloxan intravenously. The initial hyperglycemia was observed to be of the same magnitude as in control dogs receiving alloxan alone. This was followed by a transitory hypoglycemia of the same magnitude as seen in control dogs, and the final diabetic blood sugar concentration was of the same order of magnitude

in the experimental and control groups. Further administration of Regitine to the diabetic dogs did not affect the blood sugar concentration. It is concluded that a discharge of epinephrine is not responsible for the initial hyperglycemia, as some investigators suggest, and that epinephrine discharge makes little or no contribution to the diabetic blood sugar concentration. This investigation was supported in part by research grants from the National Institutes of Health, Public Health Service and Ciba Pharmaceutical Products, Inc.

Unusual effects of autonomic drugs on blockade of epinephrine and histamine responses in uterine strips from progesterone-treated cats. RICHARD E. NILSSON, ALVIN C. MCCUISTION, ABDULLAH A. ASADI, KAYE H. KILBURN AND EDWIN E. DANIEL (introduced by STEWART C. HARVEY). *Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City.*

As a result of the unexpected findings that histamine and epinephrine potentiated diphenhydramine blockade by histamine and that histamine potentiated 688A (N-benzyl-N-phenoxyisopropyl-β-chloroethylamine) blockade of epinephrine (KILBURN *et al. Federation Proc.* 11: 1952), other autonomic drugs were studied in an attempt to elucidate the nature of these uterine effects. N-isopropyl-nor-epinephrine was studied because it causes very little uterine excitatory response and is an even more potent inhibitor of spontaneous uterine activity and responsiveness to contraction-producing agents than epinephrine and histamine. It potentiated the blockade of both epinephrine and histamine and alone acted to inhibit responses to histamine. Its antihistaminic action was additive with that of diphenhydramine but was potentiated by histamine. Methacholine, which has an excitatory rather than inhibitory effect on spontaneous activity and responsiveness to contraction-producing agents, was also studied. In contrast to N-isopropyl-nor-epinephrine, epinephrine and histamine, it protected against the blockade of both epinephrine and hisatmine, although it had no effect by itself. It would appear likely that the action of these drugs is not on the receptor systems responsible for the excitant actions of epinephrine and histamine, but on a system similar to, or the same as, a system controlling spontaneous activity and responsiveness to contraction-producing agents in the uterus. Thus, sympathomimetics and histamine may act to increase and methacholine may act to decrease permeability of the muscle cell to cations (see DANIEL. *Federation Proc.* 11: 1952).

Modification by previous trauma and temperature of tolerance to tumbling shock in rats. WILLIAM C. NORTH* AND J. A. WELLS. *Dept. of Pharmacology, Northwestern Univ. Med. School, Chicago, Ill.*

It has been reported that rats subjected to previous trauma become resistant to an otherwise surely fatal amount of tumbling by the method of Noble and Collip. In our studies a significant degree of protection from normally fatal trauma (700 turns) was provided by tumbling rats at 400 turns 24 hours previously. This protection was complete if the pretrauma was given 48 hours before, and absent if given 0.5, 3, or 6 hours prior to testing. Another experiment demonstrated that the amount of preliminary trauma was of importance; 100 and 200 turns 24 hours before tumbling did not confer tolerance to 700 turns, whereas 300 and 400 turns did. Tolerance thus acquired appears to persist over a period of at least 6 months. Variation in susceptibility to other types of shock with temperature change has been reported. In our studies significant protection can be conferred by heating rats at 40°C. for 4 hours prior to tumbling, although this resistance is not apparent 24 and 48 hours after heating. The mortality of rats subjected to a temperature of 40°C. for 4 hours immediately after tumbling is significantly greater than that of unheated controls. Placing rats in a refrigerator at 4°C. for 4 hours before, or 4 hours after tumbling did not alter their mortality. Adrenalectomized animals are more susceptible than normal rats to this type of trauma, but prolonged administration of cortisone or ACTH to intact rats failed to affect their susceptibility to tumbling.

Renal excretion of radioactive digitoxin in human subjects with cardiac failure. G. T. OKITA,* F. E. KELSEY, P. J. TALSO, L. B. SMITH AND E. M. K. GEILING. *Depts. of Pharmacology and Medicine, Univ. of Chicago, Chicago, Ill.*

Highly purified radioactive digitoxin produced by biosynthesis was studied in 3 patients with cardiac failure. The drug, randomly labeled with isotopic carbon 14, was found to have a specific activity of 0.43 μc/mg. Collection of daily 24-hour urine specimens was made after the single intravenous administration of the radioactive glycoside in doses varying between 0.5 and 1.5 mg. Contrary to the findings of previous investigators, our data indicate that the major route of excretion of digitoxin in human beings is through the kidneys and not via the liver and gastrointestinal tract as previously thought. We found that 60–80% of the drug is eliminated through the kidneys either as unchanged digitoxin or its metabolites. Only 6–10% of the drug was excreted as unchanged digitoxin, while 52–72% was either chloroform or water-soluble metabolites. The more striking fact

was the long persistence of the drug in the body. Depending upon the dose, unchanged digitoxin was detected in the urine for 23–50 days, while the metabolites were excreted 31–85 days. The larger the dose the longer the drug remained in the body. There is a very rapid initial excretion of the radioactive compounds during the first 24 hours, with a marked decrease and leveling off of the excretion curve during subsequent days. Blood studies show that 1 hour after administration of the drug 10% of the radioactivity still remains in the vascular system.

Antagonistic action to the cardioaccelerator effect of ephedrine, synephrine, isuprel, tuamine and oenethyl. P. OURISSON* AND O. KRAYER. *Dept. of Pharmacology, Harvard Med. School, Boston, Mass.*

Dose response curves of the cardioaccelerator effect of ephedrine were obtained in the heart-lung preparation of the dog (HLP). Maximal acceleration was caused by a total dose of 2–3 mg. or a concentration of 1:500,000–1:300,000 of ephedrine, taking the total tissue volume of the HLP (blood, heart and lungs) to be approximately 1 liter. Using the method of Krayer (*J. Pharmacol. & Exper. Therap.* 98: 427, 1950) inhibition of acceleration by veratramine was studied quantitatively. In the presence of 3 mg. of ephedrine complete dose response curves of antiaccelerator activity were obtained with veratramine. The dose causing 50% inhibition under these conditions was found to vary between 0.26 and 0.33 mg. (average, 0.3 mg.) in 5 experiments. Atropine did not prevent or abolish the inhibitory action of ephedrine. The cardioaccelerator action of the aromatic amines synephrine and isuprel and of the aliphatic amines tuamine (2-amino-heptane and oenethyl (2-methyl amino-heptane) was antagonized by veratramine in qualitatively the same way.

Influence of anesthesia on the hypotensive and bradycardic action of veratrum. CHARLES A. PAPACOSTAS,* CLIFTON F. MOUNTAIN,* GEORGE L. MAISON AND ROBERT O. BAUER.* *Dept. of Pharmacology, Boston Univ. School of Medicine, Boston, Mass.*

Adult dogs (3 male and 3 female) were trained to lie quietly on their backs for direct measurement of femoral arterial pressure and heart rate with the Sanborn electromanometer. After basal pressure was achieved, the animal was infused for 10 minutes by vein with Veriloid in doses of ¾, 1, and 1½ times the standard 30% mean arterial pressure fall dose (MAISON *et al. J. Pharmacol. & Exper. Therap.* 103: 74, 1951). The dogs received each test dose 3 times both in the unanesthetized (U) and pentobarbitalized (A) state. (1 dose/week.)

STATE	U	A	U	A	U	A
Dose	¾	¾	1	1	1½	1½
N	18	18	18	18	18	18
MAP	121	109	135	116	122	114
SE/me	1.1	3.1	4.0	3.4	2.6	3.4
M% Fall	17	12	25	21	33	27
SE/me	3.3	2.5	3.2	2.4	2.6	2.5
MHR	78	123	84	128	78	135
SE/me	3.7	5.7	3.4	6.9	3.4	7.3
MFB	8	13	14	18	18	28
SE/me	2.3	3.8	3.5	5.0	4.0	5.9

The table presents number of observations (N), mean arterial pressure (MAP); mean percentage fall in blood pressure (M% Fall), mean heart rate (MHR), absolute mean fall in heart rate in beats/minute (MFB), and the standard error of the mean values (SE/me). Analysis of variance shows highly significant greater Veriloid hypotension in the unanesthetized state ($P < 0.001$). However, Veriloid bradycardia was greater in the anesthetized than in the unanesthetized state. This difference was not highly significant ($P = 0.025$).

Hepatic and renal copper content in experimental diabetes. EDWARD PEDRERO, JR.* AND F. L. KOZELKA. *Dept. of Pharmacology and Toxicology, Univ. of Wisconsin Med. School, Madison.*

Previous studies in this laboratory have shown that there occurs in diabetes mellitus a marked diminution in the hepatic levels of copper. The present study deals with the effect of experimentally induced diabetes on the renal and hepatic copper content in several species of laboratory animals. Experimental diabetes induced by alloxan, dehydroascorbic acid, or pancreatectomies markedly diminished both the renal and hepatic copper levels. Hypoglycemia induced by the administration of phlorhizin to rats produced a marked decrease of the hepatic copper levels without significantly altering the renal levels of copper. On the other hand, in hypoglycemias induced in rats by poisoning liver cells with hydrazine or by the administration of excessive amounts of protamine zinc insulin, there was observed a marked decrease of both renal and hepatic copper levels. Experimental ketogenic acidosis was found to have no effect on the renal and hepatic copper content in the rat except in the ketosis produced by starvation. Rats fasted for 1 week were found to have low renal and hepatic copper levels apparently due to the restricted intake of the metal.

Response of patients with myasthenia gravis to tubocurarine and decamethonium. E. W. PELI-

KAN,* J. E. TETHER* AND K. R. UNNA. *Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago, and Dept. of Medicine, Indiana Univ. Med. Center, Indianapolis.*

The thresholds of 11 patients with myasthenia gravis to the paralyzing effects of both tubocurarine and decamethonium bromide were determined and compared to the thresholds previously determined in normal unanesthetized volunteers (K. R. UNNA *et al. J. Pharmacol. & Exper. Therap.* 98: 318, 1950). Neostigmine therapy was withdrawn and the unanesthetized patients were tested on consecutive days. Aliquots of equipotent doses of the drugs (the GD95) were injected serially intravenously until objective evidence of paralysis was obtained. These minimal effective doses (in μg/kg.) were expressed as percentage of the appropriate GD95 (tubocurarine, 134 μg/kg.; decamethonium, 32 μg/kg.). Paralytic effects of both drugs affected most strongly those muscle groups involved in the disease. Threshold doses of tubocurarine in the patients averaged $2.98 \pm 2.5\%$ of the GD95 of tubocurarine; threshold doses of decamethonium in patients averaged $18.1 \pm 7.2\%$ of the GD95 of decamethonium. In normal subjects, threshold doses of both drugs were between 50 and 60% of the appropriate GD95. Thus, patients with myasthenia gravis were less resistant than normal subjects to decamethonium and to tubocurarine. However, the sensitivity of the patients to tubocurarine was enhanced to a significantly greater degree than their sensitivity to decamethonium. Unlike tubocurarine, decamethonium had a transient anti-myasthenic effect before signs of paralysis appeared. The observations on myasthenic patients further stress the differences in mode of action of tubocurarine and decamethonium and emphasize the similarity between the disease and the action of tubocurarine.

Quantitative determination of gamma hexachlorcyclohexane in animal tissue. S. ANDERSON PEOPLES. *School of Veterinary Medicine, Univ. of California, Davis.*

The widespread use of gamma hexachlorcyclohexane (I) as an insecticide has resulted in many cases of poisoning in pets and domestic animals, and has been unjustly blamed in many more. There is a great need, therefore, for a method of analysis of tissues for I which is both specific and sensitive. The methods available in the literature proved inadequate due to the low concentration of I and presence of interfering substances. The following procedure was developed to meet this need and has proven satisfactory in our hands. Twenty gm. of minced tissue are refluxed 20 minutes with 20 gm. of potassium hydroxide and 80 ml. of aldehyde-free alcohol. This procedure results in the saponification of the fats and the conversion of I to 1,2,4 trichlorobenzene. Twenty-five ml. of water is added and the digest transferred to a steam distillation apparatus. Collect 70 ml. of distillate and transfer it to a separatory funnel containing 10 ml. of Skellsolve B and shake for 2 minutes. Dry the Skellsolve layer with anhydrous sodium sulfate and read in a Beckman Model DU Spectrophotometer at both 284 and 287 mμ. The difference between the 2 readings is proportional to the concentration of trichlorobenzene in the sample and is independent of the background due to interfering substances. The actual values are obtained from a calibration curve derived from the analyses of known amounts of I. The method is quantitative on concentrations of 5 ppm. and qualitative to 1 ppm.

Mechanism of inhibition of oxygen and PAH uptake of renal slices by a cyanine dye. LAWRENCE PETERS AND MARY L. WOEHRMANN.* *Dept. of Pharmacology, School of Medicine, Western Reserve Univ., Cleveland, Ohio.*

The cyanine dye 1′ - ethyl - 3,6 - dimethyl - 2 - phenyl-4-pyrimido-2′-cyanine chloride (#863) inhibited the uptake of oxygen and of *p*-aminohippurate (PAH) by slices of rabbit kidney cortex, measured at 25°C. by the method of Cross and Taggart (*Am. J. Physiol.* 161: 181, 1950). The inhibition was readily demonstrable when the initial concentration of dye in the suspending medium was 1×10^{-3} to 1×10^{-4} molar. Marked adsorption of the dye by the slices occurred during the experimental period. The dye inhibited the respiration of a homogenate of rabbit kidney cortex at 25°C., this inhibition being unaffected by added DPN. The respiration of such an homogenate at 37.5°C. in the presence of lactate and added cofactors also was inhibited by the cyanine. The latter inhibition was reduced considerably in the presence of cresyl blue. The antagonism between cresyl blue and cyanine dye occurred also in manometric experiments with a mitochondrial preparation of rat liver in which succinate was the substrate. Further studies regarding the mechanisms of the cyanine-induced inhibition are in progress.

Physiologic and biochemic changes during xenon anesthesia. C. B. PITTINGER,* S. C. CULLEN, R. M. FEATHERSTONE AND E. G. GROSS. *Div. of Anesthesiology, Dept. of Surgery and Dept. of Pharmacology, College of Medicine, State Univ. of Iowa, Iowa City.*

Studies will be reported on the influence of xenon and the technique by which it is employed to produce anesthesia in human beings on several normal physiologic and biochemic functions, including changes in blood cell counts, oxygen and carbon dioxide tensions, hepatic and renal func-

tions, blood glucose levels, arterial tension, and electrocardiograph.

Enzyme system involved in the oxidation of carbon-21 in steroids. JOHN E. PLAGER* AND LEO T. SAMUELS. *Dept. of Biochemistry, College of Medicine, Univ. of Utah, Salt Lake City.*

One gm. of beef adrenal homogenate was incubated for 3 hours at 37°C. in 20 ml. of a Krebs Ringer bicarbonate-beef serum buffer 1:1, pH 7.4 with 2 μM of progesterone. The samples were extracted with ether-chloroform (4:1) and separated on the Zaffaroni paper chromatogram using the benzene formamide system. Known samples of adrenal steroids were run alongside the extracts; the final position of the steroids was located on these control strips and the steroid was eluted from the equivalent areas of the experimental strips. This material was estimated by analyzing for formaldehyde after periodic acid oxidation. The conversion of progesterone to adrenal steroids was poor without added co-factors. The addition of DPN alone had no apparent effect on this conversion but ATP produced marked increases in the amount of formaldehydrogenic substances in the areas of desoxycorticosterone, corticosterone-Compound S, and Compound F. Since desoxycorticosterone was among the substances produced in increased quantity, it appears that ATP activates the oxidation of the 21-methyl group of progesterone to the primary alcohol typical of the adrenal cortical hormones. Fractionation of the enzyme systems involved is now in progress.

Detoxification of mercurial diuretics by polyhydroxy thiols. A. J. PLUMMER, WILLIAM REITZE* AND F. F. YONKMAN. *Research Labs., Ciba Pharmaceutical Products, Inc., Summit, N. J.*

An extensive series of organic mercurials which were combined with various thiols has been prepared by Werner (WERNER AND SCHOLZ. *Abstracts XIIth International Congress of Pure and Applied Chemistry*. 341, 1951). These have been subjected to studies of *1*) cardiac toxicity upon injection into the left jugular vein of the dog anesthetized with Nembutal, *2*) influence on rhythmic activity of isolated rabbit ileum (Farah) and *3*) acute renal effects 24 hours after intravenous administration of graded amounts (10–40 mg/kg.) in the rat. Diuretic potency was assayed by observing the increase in urine output after the intravenous administration of the substance to a dog anesthetized with Nembutal and perfused intravenously at a constant rate with physiological sodium chloride. The purpose of the study was to reduce significantly, if possible, both the cardiac and renal toxicities while retaining a diuretic action. Thiosorbitol coupled to the mercurial was found to be an effective agent for definitely lowering the acute cardiac toxicity of several organic mercury compounds. This action was frequently, but not uniformly, associated with a decrease in the acute renal toxicity of the compound. In a series of mercurated allyl polyols, a compound in which mercurated allyl mannitol was combined with thiosorbitol produced no deleterious cardiac effect, possessed low renal toxicity and exhibited good diuretic potency.

Isolation of an epoxide metabolite from fat tissue of dogs fed heptachlor. JACK L. RADOMSKI AND BERNARD DAVIDOW (introduced by A. J. LEHMAN). *Div. of Pharmacology, Food and Drug Administration Federal Security Agency, Washington, D. C.*

While investigating the tissue storage of heptachlor (1,4,5,6,7,8,8-heptachloro 3a,4,7,7a-tetrahydro 4,7-methanoindine), a new insecticide, it was found that the fat of dogs contained a metabolically altered derivative of heptachlor. The presence of a 'detoxified' product in fatty tissue of mammals has not previously been reported. Preliminary concentration was effected by the countercurrent distribution technique of Craig using hexane and 95% methanol as the immiscible solvent pair. The middle fractions (distribution coefficient hexane/methanol = 1.35) of a 9-tube distribution were combined and put through a 22-tube distribution. The metabolite was followed by a chemical method reported elsewhere. Fractions 7–13 of the second distribution containing the metabolite were combined and put through an alumina column. After washing with hexane the metabolite was eluted with hexane containing 10% benzene. White needle-like crystals were obtained after recrystallization from methanol and water. Elemental analysis indicated the following empirical formula: $C_{10}H_5Cl_7O$. Infrared analysis showed the absence of hydroxyl groups and both infrared and the 2,4 dinitrophenyl hydrazine test showed the absence of carbonyl oxygen. The only compound which could have resulted from the oxidation of heptachlor and fit the above conditions is heptachlor epoxide. This identification was confirmed by the formation of the chlorohydrin by refluxing with 15% HCl in absolute methanol. This appears to be the first demonstration of the occurrence of epoxidation in animal tissue.

Toxicity of ANTU for the rat and its lack of dependence upon body weight. DAVID P. RALL AND WILLIAM C. NORTH (introduced by C. A. DRAGSTEDT). *Northwestern Univ. Med. School, Chicago, Ill.*

It has been reported that the toxicity of alpha naphthyl thiourea in wild Norway rats increases with the age and weight of the animals. (*Proc. Soc. Exper. Biol. & Med.* 62: 22, 1946). For example, the LD_{50} is 58.0 mg/kg. for suckling rats under

50 gm. in weight, while it is 6.2 mg/kg. for adult 450-gm. rats. This was interpreted as evidence that the sensitivity of rats to the lethal action of ANTU varies with age: the younger rats are more tolerant, the old rats more susceptible. If these data are recalculated so that the dose is expressed as mg. of ANTU/rat the LD_{50} is 2.6 ± 0.4 mg/animal for 8 weight ranges from less than 50–450 gm. with 22–82 rats in each group. This suggests that the use of the correction for body weight in expressing the toxicity of this drug is inappropriate. It seemed of interest, therefore, to determine whether such a relationship also held for laboratory albino rats. The LD_{50} for such rats weighing 100–500 gm. has been found to be approximately 1.0 mg/animal. If expressed on a mg/kg. basis, the LD_{50} varies with the weight of the animals, decreasing with heavier rats. It is concluded that the toxicity of ANTU for rats is properly expressed as a total dose of the drug/animal, and that a linear correction for body weight (mg/kg.) is falacious.

Tensilon-reversible curare agents. LOWELL O. RANDALL. *Pharmacology Dept., Hoffman-La Roche, Inc., Nutley, N. J.*

Analogs of decamethonium containing nitrobenzyl radicals in place of methyl groups on the nitrogen atoms were found to resemble D-tubocurarine in being Tensilon-reversible. Maximal potency in cats was reached in compound Ro 2-4501, N,N′-nonamethylenebis[dimethyl(*p*-nitrobenzyl) ammonium bromide] dihydrate. It had about the same activity as D-tubocurarine in the cat, but was less active in the mouse, rabbit, monkey and pigeon. It was less active on sympathetic ganglia and blood pressure but equally active on the vagus endings. Likewise, the nitrobenzyl analog of Mytolon chloride was Tensilon-reversible. This compound, Ro 2-4395, N,N′-(2,5- dihydro- 2,5- dioxo- 1,4-phenylene)bis [(3- amino-propyl) (*p*-nitrobenzyl) -diethylammonium chloride] dihydrate, was more active than D-tubo-curarine in the cat and rabbit, equally active in the mouse and pigeon and less active in the monkey. It had lower ganglionic blocking action and blood pressure effects, but equal vagal blocking action than D-tubocurarine. Analogs of succinylcholine, dipropamine and similar agents which contain the nitrobenzyl radical instead of alkyl groups on the nitrogen atoms were Tensilon-reversible. The most active compound, Ro 2-4658, γ,γ′-(*p*-phenylenedioxy) bis [diethyl(*p*-nitrobenzyl)propylammonium bromide] dihydrate, was stronger than D-tubocurarine in the cat, mouse and rabbit, equal in the pigeon but weaker in the monkey. The ganglionic blocking action and blood pressure effects were weaker than those of D-tubocurarine, but the vagal blocking action was stronger. The nitrobenzyl group when substituted for alkyl groups on the nitrogen atoms of bis-quaternary ammonium compounds appears to have the property of converting Tensilon-irreversible agents of the decamethonium type to the Tensilon-reversible agents of the D-tubocurarine type.

Urinary ammonia output in the rat as affected by the optically active forms of certain amino acids. ROBERT C. RATHBUN* AND F. E. SHIDEMAN. *Dept. of Pharmacology, Univ. of Michigan, Ann Arbor.*

Hydrated, acidotic and normal, adult, albino rats were anesthetized with pentobarbital. Their bladders were cannulated and their jugular veins exposed for injection. Urine was collected at 15-minute intervals for at least 3 successive periods, the bladder being washed at least 3 times at the end of each collection period. An amino acid was then administered, either intravenously or orally, and followed by 3 successive urine collection periods. After suitable dilution of the urine specimens, ammonia was determined on an aliquot by the Conway microdiffusion procedure and Russell's colorimetric method (*J. Biol. Chem.* 156: 457, 1947). Control experiments in normal rats showed a constant but rather low output of urinary ammonia nitrogen for periods as long as 2½ hours. Acidosis produced much higher but less constant rates of renal ammonia production. The intravenous injection of 20–30 mg/kg. of amino nitrogen as D-alanine in acidotic or normal rats resulted in an approximate doubling of the rate of urinary ammonia excretion. On the other hand, L-alanine in similar doses produced either no change or a decrease in urinary ammonia. When D- or L-leucine were administered orally at the same dose level (25 mg/kg. of amino nitrogen) increases in urinary excretion of ammonia resulted but the increase with the D-leucine was about twice as great as with the other isomer.

Excretion of potassium by the chicken kidney. BARBARA RENNICK,* CLINTON LATIMER* AND GORDON MOE. *Dept. of Physiology, State Univ. of New York Med. Center at Syracuse.*

Potassium excretion is characterized by its wide latitude, for both tubular secretion and reabsorption can occur, as was shown by Berliner and Mudge. When excretion of K exceeds 100% of the filtered load, active secretion must occur, but when less than 100% of the filtered load of K is excreted, it is not known whether the secretory process is still active. Sperber (1948) demonstrated that the fowl kidney has a functional renal portal circulation which permits blood from the limb to irrigate the renal tubules before entering the great veins. We have used the chicken in a study of the tubular excretion of K. The injection of 100 mg.

KCl i.m. into 1 thigh of a chicken increased the K excretion of the ipsilateral kidney by as much as 50%, although total excretion remained below 50% of the filtered load. It is not clear, however, whether this increased excretion is due to increased secretion, or to decreased reabsorption. Tracer amounts of radioactive K injected into 1 leg increased the excretion of K_{42} by the ipsilateral kidney to 4 times that of the control kidney even though the excretion of K remained at 20% of the filtered load. Salyrganic acid depressed K excretion in the chicken, whether excretion initially exceeded or was less than the filtered load. The effect of mercury on the excretion of K_{42} is being investigated.

General pharmacology of 4-alkoxy-β-(1-piperidyl) propiophenones. ALICE B. RICHARDS,* BENEDICT E. ABREU, EARL R. BOCKSTAHLER* AND DONALD L. WRIGHT.* *Research Dept., Pitman-Moore Co., Indianapolis, Ind.*

A series of 4-alkoxy substituted β-(1-piperidyl) propiophenones was synthesized as possible trihexyphenidyl analogs, substitutions ranging from 2–8 carbon atoms. The LD_{50}, i.v., in white mice is similar for all of these agents, varying only from 15–30 mg/kg. The maximum tolerated dose in the unanesthetized dog is 10 mg/kg. for most of these compounds. Acute toxic manifestations in the white mouse with increasing dosage include: hyperirritability, dyspnea, ataxia, spasticity and clonic convulsions terminating in fatal respiratory failure. Similar toxic signs are observed in the dog with the frequent accompaniment of salivation, nausea and emesis. All of these agents, at a dose of 2 mg/kg., i.v., in 10 seconds, in the anesthetized dog, induced cardiac slowing varying from 0 to −13% of the control and mean arterial blood pressure decreased in amounts varying from −4 to −25% of the control. No structure-action relations were apparent. Atropinization does not alter these changes nor are the cardiovascular responses to acetylcholine or epinephrine altered by propiophenones. When the maximum tolerated dose is approached in anesthetized dogs, respiratory depression, characterized by tachypnea and decreased tidal volume, appears which progresses to apnea. No significant effects on the gastroenteric tract of anesthetized dogs were observed in dose as high as 5 mg/kg., i.v. Duration of local anesthetic activity on the rabbit cornea with 0.01% concentration varied from 47–60 minutes with the N-butoxy and N-heptyloxy derivatives being the most active.

Duration of action and plasma level of thiopental in nephrectomized animals. R. K. RICHARDS, J. D. TAYLOR,* J. DAVIN* AND K. E. KUETER.* *Dept. of Pharmacology, Abbott Labs., North Chicago, Ill.*

In a previous report (RICHARDS *et al. Federation Proc.* 9:1950) we described experiments with short-acting barbiturates (Thiopental and Hexobarbital) in nephrectomized rats and rabbits. It was shown that Thiopental anesthesia was much prolonged in such animals and that a correlation existed between the time elapsed after nephrectomy and the injection of the barbiturate. Further experiments were designed to extend these findings. The effect of nephrectomy upon the M.E.D. of Thipoental was studied in rabbits, rats, and mice. In several groups of rabbits the plasma level of Thipoental was followed in the same animals prior to and several hours after bilateral nephrectomy, using both ultraviolet absorption methods and S_{35} radioactively labeled Thiopental. The relation between the blood NPN, duration of hypnotic effect, and slope of the barbiturate-plasma concentration curve will be described.

Sympathomimetic effects during high spinal anesthesia. J. A. RICHARDSON AND R. P. WALTON. *Dept. of Pharmacology, Med. College of South Carolina, Charleston.*

In fully conscious dogs, without premedication, procaine HCl was injected subdurally at the lumbo-sacral articulation. Doses of 30–65 mpk (5–10 cc. 5%) consistently produced sensory anesthesia at thoracic levels or higher and motor paralysis of hind limbs and above. A syndrome of sympathetic stimulation of variable duration occurred with reasonable regularity. This included increments in heart rate, heart contractile force and mean arterial pressure with a distinct abolition of peristaltic waves in the small gut. Circulatory stimulation was sustained for 15–30 minutes in some cases; depression developed at stages of respiratory paralysis. Abolition of peristaltic movements, determined by recording balloons in Thiry-Vella fistulae, was prompt and well sustained in some cases; equivalent doses of procaine intramuscularly did not produce such distinct effects. I.m. and subdural doses of procaine were approximately equal in circulatory stimulant effects, except the i.m. doses were more delayed and more sustained in action. Characteristic head jerking movements were somewhat less pronounced with i.m. than with equivalent subdural doses. Lower levels of spinal anesthesia (5–20 mpk) produced limited but distinct gut stimulation (increase in amplitude and frequency of peristaltic waves) in the majority of trials. In chronically adrenalectomized animals, high spinal anesthesia substantially slowed heart rate and, in dogs under pentobarbital anesthesia, high spinal anesthesia did not significantly affect heart rate or contractile force until respiratory paralysis levels produced depression. The sympathomimetic

effects in conscious animals are interpreted as being largely secondary to cerebral excitation.

Tolerance to octamethyl pyrophosphoramide (OMPA). J. A. RIDER,* L. E. ELLINWOOD* AND J. M. COON. *Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.*

Because of increasing interest in OMPA as an insecticide and as a drug for treating myasthenia gravis, additional data as to tolerance and cumulative effect have been obtained. Two separate groups of young adult male rats were injected daily with 1% aqueous OMPA s.c. In *group I* (Sprague-Dawley) 1 mg/kg. daily for 7 days produced 100% mortality. Rats which had received 0.5 mg/kg. daily for 12–56 days, however, subsequently tolerated a daily dose of 1 mg/kg. for 40 days. This tolerance was lost when the daily dose was decreased to 0.15 mg/kg. for 25 days, since 1 mg/kg. then caused 100% mortality after 5 days. Similar results were obtained from *group II* (Magurin). Though this strain seemed to be somewhat more resistant to the poison, a dose of 1.5 mg/kg. daily produced 100% mortality in 6 days. One series tolerated 1 mg/kg/day for 24 days, followed by 1.25 mg/kg. for 7 days, and 1.5 mg/kg. for 21 days. At 2 mg/kg. deaths began to occur in 2 days. In another series treated in a similar manner, 4 of 7 animals tolerated 1.75 mg/kg. for 20 days, but failed to tolerate 2 mg/kg. In a third series 2 of 7 rats were made to tolerate 2.4 mg/kg. for 17 days by gradually increasing the dose in daily increments of 0.1 mg/kg. Tolerance was lost in all animals after a rest period of 3 weeks during which time the blood cholinesterase activity returned to normal. At the end of this time 1.5 mg/kg. for 5 days produced 100% mortality. No histological changes were observed in the tissues or organs.

Formation of inorganic cyanide from aliphatic nitriles in rabbits. FREDRIC RIEDERS (introduced by CHARLES M. GRUBER). *Dept. of Pharmacology and Div. of Industrial Medicine, Jefferson Med. College, Philadelphia, Pa.*

Acetonitrile, propionitrile, *n*-butyronitrile, *n*-valeronitrile, *n*-capronitrile, iso-capronitrile, malononitrile, succiononitrile, glutaronitrile, adiponitrile, acrylonitrile and phenylacetonitrile were injected intravenously into rabbits in toxic, isomolecular quantities. At hourly intervals following the injections samples of blood from the animals were analyzed for inorganic cyanide by the micromethod of Gettler and Goldbaum (*Anal. Chem.* 19: 270, 1947) until no more cyanide could be detected. The detoxification of the inorganic cyanide to thiocyanate was followed by subsequent analyses of samples of blood by the method of Goldstein (*J. Biol. Chem.* 187: 523, 1950). There will be discussed the relationships between the rates of appearance of inorganic cyanide in the blood of the animals, the highest concentrations of cyanide which were reached, the temporal appearance and the severity of the toxic signs and the detoxification to thiocyanate. The mechanism of the oxidation of the aliphatic nitriles will also be considered.

Pharmacodynamic studies on Regitine, a new adrenergic blocking drug (Ciba 7337) 2-[-*p*'-tolyl-N-(*M*'-hydroxyphenyl)-amiomethyl] imidazoline. GWEN ROBERTS,* ALFRED W. RICHARDSON* AND HAROLD D. GREEN. *Dept. of Physiology and Pharmacology, Bowman Gray School of Medicine of Wake Forest College, Winston-Salem, N. C.*

Femoral arterial blood flow, measured by the electromagnetic flowmeter of Richardson, Denison and Green (*Circulation.* In press) and arterial pressure taken just distal to the flowmeter by a Statham pressure gauge were shown to be appreciably modified by small amounts of intra-arterial Regitine. Doses of 0.15, .1, and .015 mg/kg. resulted in very variable decreases in peripheral resistance, postinjection flows ranging from 420–85% of control flow. The magnitude of response had no apparent correlation with amount of Regitine injected, size of the animal, control blood flow or arterial pressure, type of control response to epinephrine nor with other factors such as time since last injection of anesthesia. Tachyphylaxis rapidly developed as indicated by the rapid decrease in vasodilator response to successive injections of Regitine in the same animal. Intra-arterial injection of 0.15 mg/kg. caused the vasoconstrictor response to 1 μg. of epinephrine intra-arterially (180% of control peripheral resistance) to be converted to vasodilatation (70% of control peripheral resistance). Reversal was maximum within 15 minutes and subsequent injections of epinephrine showed a gradual return to control response of epinephrine in 4–8 hours. After 0.1 mg/kg. the reversal could be elicited for a shorter time. (Supported by grant H-487(C2) from the National Heart Institute.)

A d.c. electromagnetic flowmeter for measurement of mean blood flow. R. R. ROEPKE,* J. T. LITCHFIELD, JR. AND E. M. BAILEY.* *Stamford Research Labs., American Cyanamid Co., Stamford, Conn.*

An electromagnetic flowmeter was developed for recording mean blood flow in anesthetized animals. The blood flow along with recorded pressure provides a means of measuring changes in peripheral resistance. Although the electromagnetic method has been used to measure blood flow in intact arteries (KOLIN. *Proc. Soc. Exper. Biol. & Med.* 46: 235, 1941), this appears to be feasible only in arteries such as the femoral and carotid, which can be partially isolated from the surround-

ing tissues. In the method to be described, arterial cannulation is employed to divert the blood through an external electrode unit which permits the elimination of extraneous potentials. Calomel half cells connected through saline-agar to the blood stream serve as nonpolarizable electrodes. A commercially available d.c. breaker amplifier, used in conjunction with a secondary amplifier and pen recorder, provides adequate stability and amplification. Drift of the zero-flow potential can be checked by interrupting the current to the electromagnet or, in the case of a permanent magnet, by removing the magnet from the pick-up unit, thus avoiding interruption of blood flow.

Influence of chemotherapy on the delayed deaths following extensive burns in mice. SANFORD M. ROSENTHAL, RALPH D. LILLIE AND ELIZABETH VERDER.* *Natl. Insts. of Health, Bethesda, Md.*

A method has been previously described for the study of delayed deaths following a standardized burn in mice (ROSENTHAL, S. M. *Public Health Rep.* 58: 513, 1943). The burn involves ⅔ of the body surface, and results in death from shock within 24–48 hours if no treatment is given. Death during this period can be prevented by the administration of 10–15% body weight of 0.9% NaCl. The majority of these treated animals become emaciated and die within 2–4 weeks, and the cause of these deaths has been investigated. The delayed deaths occur equally when plasma is used to prevent death from shock. Pathologic studies have not revealed changes adequate to explain them, nor have pathogenic organisms been recovered from the blood or tissues except in rare instances. These delayed deaths are related to the type of trauma, for they do not occur in mice traumatized by tourniquet application to the legs. Antibiotic and sulfonamide therapy, when given in maximum tolerated amounts, can markedly lower the mortality during the first 7–14 days, although many of the mice succumb at a later period. Streptomycin (1.5 mg/20 gm., subcutaneously, twice daily for 2 weeks or more) alone or in combination with sulfonamides or other antibiotics has so far been the most effective agent. These results suggest that infection which manifests a low susceptibility to chemotherapy may play a role in the delayed deaths following burns in mice. In the absence of antibiotic therapy no effect on the delayed deaths was observed from the administration of sodium or potassium solutions, or from ACTH and various adrenal cortical hormone preparations.

Depression of the smooth muscle activity of the small intestine of the rabbit as a measure of organic mercurial toxicity. RICHARD RUTLEDGE,* WALTER BARRETT,* JEAN FEICKERT* AND A. J. PLUMMER. *Research Labs., Ciba Pharmaceutical Products, Summit, N. J.*

A series of organic mercury thiol compounds was tested for inhibitory activity in the isolated small intestine of the rabbit according to the technique of Farah. This method consisted of suspending a piece of small intestine of the rabbit, 3–4 cm. in length, in a 50-ml. bath of Eastman-Cantoni solution. The drugs were introduced in amounts of 2, 10 and 20 μM/ml. of bath volume. After a suitable control period of normal rhythmic peristalsis, the spontaneous contractions were recorded during a 10-minute period following the application of the drug. Inhibition of peristalsis was tentatively construed as indicative of mercury toxicity. In 22 of 28 compounds, good correlation was observed between intestinal inhibition at 20 μM/ml. of bath and degree of renal damage in the rat 24 hours after the injection of 10 mg/kg. of the compound intravenously. In the remainder of the series, however, inhibition of the rabbit intestine was not correlated with tubular damage in the rat kidney. The test, despite the high percentage of correlation observed, showed that intestinal inhibition at a concentration of 20 μM/ml. does not necessarily imply the occurrence of renal tubular damage in the rat after the intravenous injection of 10 mg/kg. of a compound.

Experiments on the histamine-thyroid-parathyroid-adrenal relationship. RAYMOND R. SACKLER (introduced by C. Co TUI). *Creedmoor Inst. for Psychobiologic Studies, New York City.*

The statistically significant increase in histamine tolerance produced by thyro-parathyroidectomy supports the hypothesis that elimination of thyroid and/or parathyroid from endocrinium releases greater operative effect of histamine antidynes (i.e. antagonists such as adrenal hormones). Findings suggest counter-proof for postulated thyroid-adrenal antidyne relationship previously revealed by demonstration of reduction of histamine tolerance by thyroid administration. Increase in histamine LD_{50} in females is twice that found in males following thyro-parathyroidectomy suggesting *1*) a quantitative and/or qualitative difference between thyroid and/or parathyroid function in the female and the male rat; and/or *2*) a difference in the interactions and effects of the different gonadal hormones on thyroid and/or parathyroid function; and/or *3*) a greater adrenal-antidyne role by the male than by the female hormones; and *4*) a physiologic hypothesis for the higher incidence of thyroid dysfunction in the female than in the male.

Administration of pharmacological agents as a protective measure against the lethal effects of x-irradiation in mice. PAUL R. SALERNO,* PAUL A. MATTIS AND H. L. FRIEDELL.* *Atomic Energy*

Med. Research Project, Western Reserve Univ., Cleveland, Ohio.

Numerous agents have been examined for their effect against irradiation damage. Hollaender (*Proc. Soc. Exper. Biol. & Med.* 77: 636, 1951) has reported a reduction in the radiation sensitivity of *E. coli* B/R in the presence of certain alcohols and glycols. We have been investigating the effect of similar compounds on the radiation mortality in mice. The intraperitoneal administration of glycerin (3.0 cc/kg.), 5–15 minutes before exposure to 600 r, reduced the 30-day mortality in CF_1 female mice. Only 3 of 48 control mice survived, whereas 29 of 48 glycerin-treated animals were alive at the end of 30 days. Propylene glycol (5.0 cc/kg.), under similar conditions, was less effective, 19 of the 48 animals surviving the 30-day period of observation. Several other glycols, alcohols and their amino- or sulfhydryl-substituted derivatives have been found to modify the sensitivity of mice to x-irradiation. Metabolic intermediates, including pyruvate, lactate, acetone, and acetate have been tested in an effort to determine the mechanism by which the altered radiation sensitivity is produced. Since it is known that compounds of this nature require oxygen for their metabolism, it seems likely that the protective effect is dependent upon this aspect of their metabolic alteration. In the case of bacteria, Hollaender and others have suggested that the mechanism of protective action of similar oxidizable substances is due to a reduction of the oxygen concentration within cells or in their environmental media. Possible mechanisms of action in animals will be discussed.

Inotropic activity of blood serum on myocardium compared with that of cardiac glycosides. WILLIAM T. SALTER AND RICHARD M. TAYLOR.* *Labs. of Pharmacology and Toxicology, Yale Univ. School of Medicine, New Haven, Conn.*

The pioneer work of Bowditch and A. J. Clark indicated a stimulant action of blood serum on heart muscle. The activity resides, *not* in the crude globulin fraction, but in the albumin-mixture, even in the more pure preparations of E. J. Cohn. After 4 months in solution at 4°C. the activity decreases ½; but lyophilized albumin remains highly active over a year. The serum of young healthy adults is more active than serum from older people. Maximal values, measured in diluted serum, are equivalent to some 30 μg/100 ml. of ouabain (hydrated). In whole serum, only ⅓ of the total activity is manifested (i.e. free). Like ouabain, the albumin fraction (and the whole serum) shows synergism with calcium ions. The dosage-response curve for albumin resembles that for ouabain; and the onset, of action is similar in time. To date, however toxic rigor has *not* been observed. A diagram has been formulated to measure this activity in ouabain-equivalent units.

Effect of hypophysectomy on DCA-induced hypertension in rats. F. J. SAUNDERS,* F. J. MCDONOUGH,* NANCY WAHLGREN* AND D. M. GREEN. *Div. of Biological Research, G. D. Searle and Co., Chicago, Ill.*

Two groups of male rats were implanted with 20 mg. desoxycorticosterone acetate (DCA) pellets and given 0.86% sodium chloride solution as their drinking fluid. One group was hypophysectomized before implantation. Blood pressures remained normal throughout the period of observation. The second (nonhypophysectomized) group rapidly developed hypertension which persisted for months. At a time when the blood pressure averaged 184 mm. Hg systolic, a portion of this second group was hypophysectomized. One day postoperatively, the average blood pressure (30 rats) was 152 mm. Hg. Twenty-four days post operatively, the pressure (10 surviving rats) was 133 mm. Hg. The remainder of the second group was sham-hypophysectomized. Three days postoperatively, the average blood pressure (15 rats) was 162 mm. Hg. Twenty-four days postoperatively, the pressure (14 surviving rats) was 204 mm. Hg. The hypophysectomized animals showed significantly lower testis, seminal vesicle, adrenal and kidney weights but no difference in heart weight as compared to the sham-operated animals.

Nephrotoxic action of tetrathionate in the rabbit. JACK P. SAUNDERS,* HAROLD I. GREEN* AND J. H. WILLS. *Pharmacology Branch, Chemical Corps Med. Labs., Army Chemical Center, Md.*

Renal function tests were performed on rabbits before and after intravenous administration of doses of sodium tetrathionate ranging from 10–100 mg/kg. The 10-mg/kg. dose had no significant effects on glomerular filtration (inulin clearance), on tubular transport of glucose and on tubular transfer of chloride. It did decrease the tubular excretory mass (hippurate) by about 20%. Higher doses of tetrathionate produced decreases in all these variables. The 100-mg/kg. dose lowered inulin clearance to about 10% of the control value, tubular transport of chloride to about 20%, tubular excretory mass to 5% and tubular transport of glucose to 0. The data suggest that low doses of tetrathionate affect primarily the proximal tubule, with gradual involvement of the rest of the nephron as the dosage is increased.

Effect of exercise on curarization. JOHN L. SCHMIDT,* HAROLD F. CHASE AND GOVENOR WITT.* *Dept. of Pharmacology, Western Reserve Univ., and Dept. of Pharmacology, Abbott Labs., North Chicago, Ill.*

Strenuous exercise for 2 minutes doubles the

sensitivity of rabbits to D-tubocurarine as determined by the rabbit head-drop assay. There is a relation between the degree and the duration of exercise and the resultant effects on curarization in rabbits. After strenuous exercise for 2 minutes the control level of sensitivity is not restored on the average until after 40 minutes of rest. A parallel between these changes and changes in blood lactic acid content following exercise has been determined. A correlation of the exercise effect on curarization and changes in blood pH will be reported. The effect of the above humoral changes on the sensitivity to D-tubocurarine when produced by means other than exercise (e.g. acid infusion, epinephrine injection) will also be reported, as will be the action of cholinesterase inhibitors. Efforts to localize the site of action have been made in isolated, paired, nerve-muscle preparations of the hind limbs of rabbits with intact circulation, and the results of these studies will be presented. Several interesting parallels may be drawn between the results of the above studies and certain findings in cases of myasthenia gravis.

Polarizing forces of the muscarinic moiety. F. W. SCHUELER. *Dept. of Pharmacology, State Univ. of Iowa, Iowa City.*

Using a method similar to that developed by Price (*Mechanisms of Reactions at Carbon-Carbon Double Bonds*, New York: Interscience 1946) the polarizing forces (P.F.) of the moiety components of well known muscarinic drugs were calculated and correlated with their depressor potency. For example, the $\left(\begin{matrix}O\\ \|\\ -O-C-\end{matrix}\right)$, P.F. = −0.32, of acetylcholine upon substitution by (—O—CH_2—), P.F. = −1.21; (—CH_2—CH_2—), P.F. = +0.77; and $\left(\begin{matrix}O\\ \|\\ -CH_2-C-\end{matrix}\right)$ P.F. = +.089 illustrates the fact that the *absolute* deviation of the P.F. of the substituent group from the P.F. of the parent group in acetylcholine is inversely correlated with the potencies of the above agents in the order 1 (for acetylcholine) $\frac{1}{3}$, $\frac{1}{6}$, 1/70, respectively. Likewise the replacement of the linkage $\left(\begin{matrix}O\\ \|\\ -O-C-CH_3\end{matrix}\right)$ by (—I), P.F. = −0.39, (—Br), P.F. = −0.55, (—Cl), P.F. = −.068, and (—OH), P.F. = −1.46, are correlated with the potencies in the order 1 (for acetylcholine) 1, 1/10, 1/20, 1/200. A similar relationship holds for the positive end of the acetylcholine molecule where (—$N^+(CH_3)_3$) P.F. = +3.21 on demethylation yields (—$N^+(CH_3)_2H$), P.F. = +3.02, (—$N^+H_2CH_3$), P.F. = +2.88, and (—N^+H_3), P.F. = +2.64 are correlated with potency in the order 1 (for acetylcholine), 1/50, 1/1500, 1/2000. The estimation of polarizing forces by such a method allows a more rigorous evaluation of the character of moiety constituents than vague reference to groups of 'high' or 'low' polarity and functionally expresses the effect of 'minor' changes such as the degree of methylation of nitrogen.

Effects of intravenous infusion of potassium on liver glycogen. R. A. SEIBERT,* R. A. HUGGINS AND R. SAXTON.* *Dept. of Pharmacology, Baylor Univ. College of Medicine, Houston, Tex.*

An intimate association between potassium transfer and carbohydrate metabolism has been suggested by many authors. Fenn has reported that glycogen is deposited in the liver with the same amount of water, potassium, and acid-phosphate as is found with other liver constituents. In dogs anesthetized with either morphine or sodium barbital, the intravenous infusion of maximum sublethal amounts of isotonic potassium chloride resulted in a 60% decrease in liver glycogen. The blood glucose of control and experimental animals anesthetized with morphine was elevated at the start of the experiment but fell to normal values during the 4 hours of the experiment in spite of the fact that glycogen was being lost from the liver. In the barbitalized control dogs, the blood glucose was normal at the start but slowly increased to a maximum at 2 hours; whereas, the experimental dogs showed no increase in blood glucose. At no time did the blood glucose account for the glycogen lost from the liver. The blood potassium of the experimental animals was, of course, increased over that of the control dogs. If the liver potassium was calculated on a dry weight basis, there was an increase of 18% in the experimental animals. This increase in liver potassium concentration may indicate that glycogen and potassium can move independently in the liver.

Technical factors influencing the permeability of synovial membrane. JOSEPH SEIFTER, DAVID H. BAEDER* AND ALBERT BLUMENTHAL.* *Wyeth Inst. of Applied Biochemistry, Wyeth, Inc., Philadelphia, Pa.*

More than 5000 experiments on 1658 rabbits have made possible a statistical analysis of the method described by Seifter, Baeder and Begany (*Proc. Soc. Exper. Bio. & Med.* 72: 277, 1949) for measuring the permeability of synovial membrane. It has been found that control of many technical factors is necessary for success of the method. These are *1*) care of the animal, *2*) catheterization, *3*) use of only the talocrural articulation, *4*) accuracy of injection of the phenolsulfonpthalein (PSP) into the talocrural articulation, *5*) mechanical restriction of the blood supply to the joint area, *6*) vasoconstriction, *7*) vasodilation. Any of

these influence the uptake of PSP sufficiently to alter the characteristic clearance curve. Accurate injection of the PSP into the talocrural articulation is critical; and failure on the first attempt renders the animal useless for the experiment. Standardization of the 1658 rabbits for control purposes was done in 16 groups and statistical analysis shows a standard deviation = 6.01 %, standard error = 1.93 %. When 1 rabbit was chosen at random from each of the 16 groups standard deviation = 8.03 %, standard error = 2.01 %. Based on these figures the effect of a compound on permeability can be determined in 2 previously standardized animals. The standard deviation was 1.1 % and the standard error was 0.35 % in 10 rabbits receiving cortisone acetate intramuscularly. In 15 rabbits receiving 21 acetoxypregnenelone intramuscularly standard deviation = 0.71 %, standard error = 0.02 %. The statistical data confirm that with proper technique the method is precise and accurate for measuring synovial membrane permeability.

Ten-hour preparation of pure, highly polymerized thymus nucleic acid. NORMAN S. SIMMONS, STELLA CHAVOS AND H. KENNETH ORBACH (introduced by RICHARD F. RILEY). *Atomic Energy Project, School of Medicine, Univ. of California, Los Angeles.*

A simple rapid method has been devised for the preparation of thymus nucleic acid in approximately ten working hours at 0° and at pH 7.0. Fresh thymus tissue is homogenized 5 times in cold citrate-saline. The nuclear residue is taken up in 1.5 NaCl −0.01 M citrate, stirred one half hour and the proteins precipitated by the addition of sodium xylene sulfonate. The precipitate is removed by filtration and the nucleate precipitated with 0.35 volumes of 98% isopropyl alcohol. The nucleate is redissolved in water, brought to 0.3 M with NaOAc and reprecipitated with 0.53 volumes of isopropyl alcohol. This is repeated 3 times more. All reactions are carried out at 0°. Yield is 1.5 gm/100 gm. tissue. Analysis: C 35.4 %, H 3.4 %, N 16.0 %, P 9.4 %, Na 6.9 %. All preparations were ultracentrifugally and electrophoretically homogeneous at pH 4.5–7.5. Viscosity studies indicate an intrinsic viscosity of 30 in PO_4-NaCl buffer pH 7.0 and 0.2 ionic strength. Preliminary molecular weight studies indicate a reduced sedimentation constant of about 20×10^{-13} and a diffusion constant of below 0.40×10^{-7}, indicating a narrow distribution of molecular weights above 2.5×10^6 but below 4×10^6. The analytical results closely approximate those for a statistical tetranucleotide.

Toxicity of heated proteins. ANDRÉ SIMONART (introduced by ISAAC STARR). *Dept. of Pharmacology, Univ. of Louvain, Louvain-Belgium.*

After establishing that the principal cause of death in experimental burns is the resorption of toxic material, an attempt was made to reproduce the toxemia by injecting heated serum or plasma. The experiments were mostly performed on frogs. It appeared that all the examined serums and plasmas (rabbit, ox, horse, pig, human), heated above 70°, produce in high doses a lethal intoxication with symptoms of burn toxemia. All heated solutions of various protein fractions of bovine serum: crystallized serum albumin, crystallized gamma globulin, fraction V, fraction IV1, fraction IV4, II and III together, produce a similar intoxication. The main symptoms are: general edema, petechiae, and gastrointestinal hemorrhage. Autopsy shows dilatation and congestion of stomach and intestine with petechiae in various organs including muscle. When serum or protein solutions are injected after heating to a pH between 4 and 7 the hemorrhagic symptoms become predominant. Rapidly fatal intoxications (death after 1–3 hours) produce little bleeding. Heating between pH 7 and 10 produces a nearly equivalent intoxication with little bleeding. The highest toxicity was found at pH 4.5. Dialysis against various fluids and on different membranes gave irregular results. Intoxications were produced by dialysates obtained on collodion or on cellophane (Visking). The subcutaneous injection in rabbits of large quantities of heated horse serum produces fatal intoxication in 1–10 days. The smallest effective dosage was 40 cc/kg. The main symptoms were: lack of appetite, loss of weight and congestion of the G-I tract. The injection of heated solutions of crystallized bovine serum albumin (3 gm/kg.) produced similar fatalities.

Biological and physico-chemical methods in the standardization of veratrum ester alkaloids. FLOYD R. SKELTON, MARJORIE BECK AND GORDON A. GRANT (introduced by R. A. WAUD). *Research and Biological Labs., Ayerst, McKenna and Harrison, Ltd. Montreal, Quebec, Canada.*

A multiple dose-response method for the estimation of hypotensive potency of veratrum ester alkaloids has been developed, using the arterial blood pressure fall in anesthetized, normotensive dogs as the criterion of response. The method differs from the equipotent-dose assay of Maison and Stutzman (G. L. MAISON AND J. W. STUTZMAN, *Arch. Intern. Pharmacodynamie*, 85: 357, 1951) in that at least 3 dose levels of standard and unknown are administered intravenously to groups of 6 or more animals. Thus the slopes of the regression lines are obtained and potency estimated therefrom. Technical details and statistical evalu-

ation of the method will be presented. Biological assays being time consuming a more rapid procedure for assessing the activity of veratrum ester alkaloids was highly desirable. Since their hypotensive potency appears closely related to the degree of esterification, a method was devised by which the number of ester linkages could be readily estimated. This consists in determining the the optical density of the carbonyl band at 1736cm^{-1}. By comparison with known standards, it is possible to express the result in terms of the equivalent degree of esterification (G. PAPINEAU-COUTURE AND G. A. GRANT, *Chemistry in Canada*, Jan. 1952). An interesting parallelism has been found between the biologically estimated hypotensive potency of veratrum ester alkaloids and their 'ester number' as determined above. The infrared analytical procedure has been of value in fractionation studies concerned with isolating individual ester alkaloids, and approximating the hypotensive potency of mixed alkaloidal preparations prior to biological assay.

Cardiovascular and renal effects of nor-epinephrine and its use as a vasopressor agent in the treatment of severe shock unresponsive to other therapeutic agents. JAMES A. SKELTON,* LEWIS C. MILLS* AND JOHN H. MOYER. *Depts. of Medicine and Pharmacology, Baylor Univ. College of Medicine and Cardiac Clinic, Jefferson Davis Hosp., Houston, Tex.*

The cardiovascular and renal effects of norepinephrine were studied in 23 normal subjects and 17 patients in shock not responding to the other therapeutic measures. The drug was given subcutaneously (0.6 μgm/kg.) to 15 normal subjects on whom renal function studies were not done. It was given by continuous intravenous infusion to 8 normal subjects on whom renal function studies were done and to the 17 patients in shock. In the normal subjects given the drug subcutaneously, a vasopressor effect without subsequent vasodilatation was noted which lasted about 30–45 minutes. The pulse rate decreased consistently. The vital capacity, circulation time, respiratory rate, blood sugar, and hematocrit were not altered significantly. When given by continuous infusion, the mean blood pressure increased 30 %, whereas the renal plasma flow decreased 40 %. Glomerular filtration rate decreased 8 %, whereas TmPAH was unaltered. Subjective symptoms which are so marked with epinephrine were minimal and frequently were entirely absent with nor-epinephrine. The 17 patients in shock consisted of 6 cases of myocardial infarction, 4 patients with overwhelming infection, 2 patients in heart failure with peripheral vascular collapse, 4 postoperative patients, and one in peripheral vascular collapse following intravenous aminophylline. The systolic blood pressure was below 60 mm. for 30 minutes or more in all of the patients before the infusion was started and in a number the blood pressure was unobtainable. All but 1 patient responded by an increase in blood pressure to within normal limits. Nine of the patients recovered completely and were discharged from the hospital. Although all of the patients were anuric during the shock stages, none showed residual evidence of significant impairment of renal function.

Action of perimidine on the central nervous system. IRWIN H. SLATER AND D. E. LEARY.* *Divs. of Orthopedic Surgery and Pharmacology, Univ. or Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Goodman *et al.* (*Federation Proc.* 2: 80, 1943) reported that benzimidazole is a potent depressant. Unna and his co-workers reported on the activity of many compounds patterned after this type of fused ring structure including several derivatives of benzothiazole. We have studied perimidine (perinaphthimidazole) which bears a generic relation to the above compounds. The mean effective dose for loss of righting reflex (ED_{50}) in mice after intraperitoneal injection was 35.6 ± 5.9 mg/kg. The mean lethal dose was 126 ± 25.2 mg/kg. The duration of paralysis was short. Orally the ED_{50} was 237 ± 32.5 mg/kg. The mice rapidly recovered from the depressant effects, but death occurred as late as 48–72 hours after recovery of righting reflex. We have not studied the cause of this late toxicity. The compound was not effective against Metrazol, strychnine, electric shock convulsions. In spinal cats and in cats anesthetized with Dialurethane (0.66 mg/kg.) perimidine doses of 5–10 mg/kg. caused obtundation of the flexion reflex without effecting the knee jerk or myoneural transmission. In decerebrate cats, 10 mg/kg. intravenously caused prompt relaxation of the rigidity as judged by clinical examination and electromyography. Perimidine appears to be another of the large group of compounds which cause a selective depression of multisynaptic reflex arcs. Because of its extremely short duration of action it would not appear to be of clinical interest.

Toxicological studies on the zinc and disodium salts of ethylene bisdithiocarbamate (dithanes Z 78 and D 14). R. BLACKWELL SMITH, JR., J. K. FINNEGAN, P. F. SAHYOUN,* H. B. HAAG AND P. S. LARSON. *Depts. of Pharmacology and Surgery, Medical College of Virginia, Richmond.*

The acute oral LD_{50}'s in rats of the zinc and disodium salts of ethylene bisdithiocarbamate are >5200 mg/kg. and 395 ± 12 mg/kg., respectively. The zinc compound has less than $\frac{1}{10}$ the goitrogenic potency reported for the disodium salt.

Dietary levels of 10,000 ppm of the zinc salt are required to produce unequivocal microscopic pathology of the rat thyroid within 30 days, although an upward trend in gland weights appears at lower levels. This lesser goitrogenic potency is apparently due to the fact that only 11–17% of the zinc compound is absorbed from the gastrointestinal tract. Neither of the salts is a notable primary irritant to the skin of man. Individuals may be sensitized to skin contact with the disodium salt, but no evidence of sensitization was obtained with the zinc compound. The feeding to rats of dietary levels of zero, 500, 1000, 2500, 5000 and 10,000 ppm of the zinc salt for a period of 2 years did not affect mortality in the male. In the female there was apparent increased mortality at the 5000 and 10,000 ppm levels. A tendency toward diminished growth appared in both sexes at the 10,000 level. Adult dogs have been fed the zinc compound at dietary levels of 20, 200 and 10,000 ppm for 1 year without adverse effect on body weight. Dogs fed 10,000 ppm showed a definitely increased thyroid weight to body weight ratio. Histopathologic studies are in progress.

Dextran tolerance studies. J. N. Spencer and H. G. Payne.* *Pharmaceutical Research Div., Commercial Solvents Corp., Terre Haute, Ind.*

The introduction of dextran as a plasma volume expander has raised the question as to the tolerance of the animal body for this material on repeated administration. Accordingly adult dogs were administered intravenously 20 ml/kg. of 6 % dextran solution for 30 days, 2 animals being treated daily and 2 every other day. No ill effects were produced by the treatment. In animals treated daily, the blood dextran averaged 250 mg. % and urine dextran 260 mg. %, 24 hours after dextran administration and those treated on alternate days 110 mg. % and 100 mg. %, respectively, 48 hours after administration. No dextran was detected in the cerebrospinal fluid and the blood sugar and NPN was not altered by the dextran treatment. Hemodilution was evidenced by a progressive decrease in the erythrocyte count amounting to 15 % by the 30th day. This was associated with a concomitant decrease in the hemoglobin and hematocrit. More striking was the decrease in plasma proteins. This reached a maximum of 18 % in the dogs receiving dextran every other day and 34 % in those treated daily. Within 7 days after cessation of therapy, however, the blood and urine were free of dextran and the erythrocyte count, hemoglobin, hematocrit and plasma proteins had returned to normal. The microscopic evaluation of the tissues of these animals will be reported.

Estimation of endothal in urine. Stanley E. Srensek, William D. Harkness and Geoffrey Woodard (introduced by A. J. Lehman.) *Div. of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*

In studying the absorption, distribution and excretion of Endothal (sodium 3,6-endoxo-hexahydrophthalate), a selective defoliating agent, it was necessary to develop a method for its detection in biological fluids and tissues. Since no adequate chemical method was available, advantage was taken of the observation reported to us by the manufacturer of Endothal that it inhibits germination of certain seeds. Seed germination tests were carried out in Petri dishes containing a filter paper wetted with 5 ml. of solution. Germination of wheat, millet, clover, buckwheat, vetch, and rye grass was inhibited by 10 μg or less/ml. of solution. Of these, millet was chosen since it was one of the most sensitive to Endothal while remaining relatively insensitive to sodium ions. Estimation of Endothal in an unknown is made by comparing the graded response to various dilutions of the unknown with the graded response to increasing dilutions of an Endothal standard. Tentative identification of Endothal can be made by the differential response of a variety of seeds to an unknown as compared to their response to Endothal. By the use of this method, it appears that 66% of a 2-mg. subcutaneous dose in rats is excreted in the urine in 24 hours; 33% within the first 5. Similarly less than 15% of a 2-mg. oral dose is excreted. The application of this method to other biological material will be reported.

Influence of the posterior pituitary on the pressor action of epinephrine. G. W. Stavraky and R. J. Oliver.* *Dept. of Physiology, Univ. of Western Ontario, London, Canada.*

In 61 cats, section of the spinal cord below the medulla and severance of the vago-sympathetic trunks in the neck was carried out. The pressor responses to double injections of 2.5–25 μg. of epinephrine hydrochloride (Parke Davis) were studied under conditions of carefully controlled pulmonary ventilation. Augmentation of the pressor responses to epinephrine was observed during stimulation of the central ends of the vagi nerves and during asphyxiation of the head brought about by clamping of 1 vertebral and 2 common carotid arteries. Clamping of the innominate veins reduced the effectiveness of the injections of epinephrine. A similar reduction in the effectiveness of epinephrine took place after hypophysectomy or when the animal was decapitated. Subminimal quantities of posterior pituitary extracts (0.1–0.25 iu Pituitrin, Burroughs & Wellcome, or Pitressin, Parke Davis) given intravenously in advance of the injections of epinephrine,

or concurrently with them restored the full effectiveness of the epinephrine after hypophysectomy. Under the influence of postpituitary stimulation a greater augmentation was observed of the response to the first injection of epinephrine as compared with the second one. It is concluded that under the prevailing experimental conditions the agent involved in the modification of the pressor responses of epinephrine was the pressor principle of the posterior pituitary gland and that the posterior pituitary besides enhancing the irritability of the vascular bed to epinephrine may retard the recovery of the contractile tissues from a preceeding contraction.

Intravenous choline therapy. F. STEIGMANN, R. FIRESTEIN* AND J. DE LA HUERGA.* *Hektoen Inst. for Med. Research of Cook County Hosp. and Dept. of Internal Medicine, Univ. of Illinois College of Medicine, Chicago.*

Recently, choline has become widely used in liver disease, in daily oral doses of 3–9 gm. with few toxic effects. Its parenteral use has been considered dangerous, because of its similarity in action to acetylcholine. To study possible reactions of intravenous choline, 12 patients were given 2 gm. choline base in 500 cc. of 5% glucose during 2 hour periods and observed for changes in the electrocardiograms, blood counts, urine, blood pressure, and pulse (the latter taken every 15 minutes); also for any symptoms of dizziness, nausea, palpitation, restlessness, flushed skin and vomiting. Only 1 of the 12 patients showed definite ECG changes; his P-R interval of 0.18 changing to 0.3 seconds. There were no significant changes regarding hemoglobin, RBC, WBC, and polymorphonuclear cells and only slight inconsistent changes (increase or decrease) in the eosinophiles, lymphocytes and monocytes. The blood pressures showed a slight initial drop with a later rise to their original levels in some. The pulse rate reacted similarly. The urine remained negative for albumin and sugar. Choline content was about 10% of the dose given in the 0–4 hours and traces in the 4–24 hours specimens. Subjectively, there were only few symptoms (warmth, lightheadedness) in 11 of the 12 patients; 1 patient with cirrhosis had a chill suggesting the innocuousness of such doses of intravenous choline.

Antihistaminic drugs in treatment of postgastrectomy dumping syndrome. EMANUEL STEIN (introduced by MICHAEL G. MULINOS). *New York Med. College, New York City.*

Symptoms of the early type of postprandial dumping syndrome following gastrectomy show much resemblance to acute histamine poisoning. It is, therefore conceivable that the dumping syndrome is caused by a sudden liberation of excessive amounts of histamine from the gastrointestinal tract under the stimulus of food intake. On the basis of this hypothesis 4 patients having the early postprandial dumping syndrome were treated by antihistaminic drugs. Two of them received tripelennamine and 2 chlorprophenpyridamine. The antihistaminics were administered 1½ hours before ingestion of food. All 4 subjects responded well to this treatment. Postprandial epigastric distress, flushes, tachycardia and diarrhea disappeared promptly and all 4 persons were markedly relieved by this treatment. One of the subjects who showed symptoms both of the early and late types of postprandial dumping syndrome with occasional hypoglycemic signs improved promptly under the effect of tripelennamine. With the subsiding of early postprandial symptoms the hypoglycemic syndrome also disappeared: One of the subjects who responded well to chlorprophenpyridamine was previously resistant to all other forms of treatment, including bilateral vagotomy. Further studies on this problem are in progress.

Action of procaine amide on ventricular tachycardia produced by coronary artery ligation. HAROLD STERN,* JOHN YELNOSKY* AND BYRON B. CLARK. *Pharmacology Dept., Tufts College Med. School, Boston, Mass.*

A long-lasting ventricular tachycardia was produced in the dog by ligation of the anterior descending coronary artery in 2 stages (HARRIS *et al. Circulation* I: 1318, 1950). Procaine amide administration was started on the first postoperative day without anesthesia, usually in a dose of 20 mg/kg. every 30 minutes i.v. to a total of 60–120 mg/kg. for each test day. In every case (6 dogs) the total and the ectopic ventricular rate was slowed, and in most tests temporary reversion to normal sinus rhythm was accomplished for varying intervals of a few minutes to several hours depending upon dosage. Blood pressure was also recorded on 2 animals in which therapeutic effects were obtained on the arrhythmia without appreciable change in intra-arterial pressure. There appears to be a close correlation between plasma levels of procaine amide and therapeutic response. Experiments are in progress to determine an optimal dosage schedule. The temporary restoration of sinus rhythm resulting from a single dose of the drug and followed by return to the initial ventricular rhythm suggests that the technique may be useful for screening drugs for antiarrhythmia action. Since the animals are unanesthetized an estimate of toxicity can be made at the same time.

Hemorrhagic shock. JOS. G. STRAWITZ,* W. C. LEE,* E. R. SCHMIDT* AND O. S. ORTH. *Depts. of Surgery and Pharmacology, Univ. of Wisconsin Med. School, Madison.*

The efficacy of 1-ethane sulfonyl (4-ethyl) piperazine in prolonging the survival time of dogs subjected to irreversible hemorrhagic shock has been determined. The animals were anesthetized with pentobarbital sodium 30 mg/kg. intravenously and heparin 2 mg/kg. was given to prevent clotting. Mean arterial blood pressure was determined directly from the femoral artery with a mercury manometer and recorded graphically. Using paired dogs of the same sex, approximately the same age and weight, the blood pressures were lowered simultaneously to about 40 mm. Hg by rapid bleeding from the femoral arteries. Subsequent small withdrawals of arterial blood were made as necessary to maintain the hypotensive levels. After such a hypotensive level had been maintained for at least 30–60 minutes, 50 mg/kg. of 1-ethane sulfonyl (4-ethyl) piperazine was administered in 5 cc. of saline solution intravenously in 10 minutes to 1 animal of the pair. The control animal was given a similar volume of saline. The administration of drug usually produced a fairly pronounced fall of blood pressure. There was recovery from this decline to the previous hypotensive level if the drug was given within the first hour of hypotension. Upon injection after 1½ hour or more, the pressure remained depressed and death usually was hastened. Observation of both animals at the mean blood pressure level of not above 40 mm. Hg was continued until death ensued. The drug was considered to be effective if the treated animal survived the control by a significant period of time. Post-mortem examinations were performed.

Effect of nitrogen mustard on alloxan diabetes. FRANK M. STURTEVANT, JR.,* FRANCIS J. SAUNDERS* AND D. M. GREEN. *Div. of Biological Research, G. D. Searle and Co., Chicago, Ill.*

Alloxanized male rats were injected daily with 0.01–0.20 mg/kg. intravenously of methyl bis (β-chlorethyl) amine for periods of 5, 11 and 33 days. Weekly body weights and daily urine volumes and glucose excretions were recorded. Response was measured by percentage decrease in daily glucose excretion, the excretion of the 3 days preceding treatment being compared to that of the 3rd, 4th and 5th days of treatment. Nitrogen mustard reduced glycosuria in such a manner that a log dose-response curve could be drawn and compared to a similar curve for protamine zinc insulin. The log doses of mustard and insulin were linearly related through equivalent responses. The length of mustard treatment determined the duration of reduced glycosuria. Administration of nitrogen mustard to 3 human diabetic patients in doses of 0.10 mg/kg/day for 5 days was without influence on blood glucose level or urinary glucose excretion.

Effect of the liver on the biotransformation of *d*- and *l*-methadone. CHEN-YÜ SUNG* AND E. LEONG WAY. *College of Pharmacy and Div. of Pharmacology and Exptl. Therapeutics, School of Medicine, Univ. of California, San Francisco.*

It was previously established that the liver is the chief organ for metabolizing *d, l* methadone (SUNG, C. Y. AND WAY, E. LEONG. *J. Pharmacol.* 98: 72–76, 1950). However, it has not been established that the *d* and *l* forms have identical metabolic pathways. Since they differ greatly in their pharmacologic properties, studies were initiated to ascertain if the liver would differentiate between the 2 isomers with respect to rate or manner of degradation. When approximately 200 mg. of rat liver slices were suspended in 3 cc. of Kreb-Ringer solution containing 20 μg. of *d*- or of *l*-methadone in the presence of oxygen, the amount of each form recovered after various time intervals was approximately the same. The optimal pH for the reaction was found to be the same for both isomers, being between 7.5 to 8.5. Hydroxylamine, azide cysteine, ascorbic acid, glutathione, fluoride, cyanide, and arsenite all inhibit the reaction to varying degrees, but none of the inhibitors exhibited any differential effect on the rate of degradation of the *d* and *l* forms. It appears, therefore, that *d*- and *l*-methadone have certain metabolic pathways which are identical. (Supported in part by the National Institutes of Health, Bethesda, Maryland.)

Effects of liver injury and nephrectomy on the anticonvulsant activity of trimethadione. EWART A. SWINYARD, R. E. BRINEY,* G. A. BUCHANAN,* D. A. BURNETT,* KEITH FORSYTH,* HAROLD OLPIN* and LOUIS S. GOODMAN. *Depts. of Pharmacology, College of Pharmacy and College of Medicine, Univ. of Utah, Salt Lake City.*

The ED_{50} of trimethadione was determined at 2, 5 and 12-hour intervals in normal, carbon tetrachloride-treated and nephrectomized rats; the end point employed was abolition of the tonic extensor component of maximal electroshock seizures. The drug was administered intraperitoneally at times selected to permit testing at 36–48 hours after a single subcutaneous dose of carbon tetrachloride (2 cc/kg. of a 50% solution in peanut oil) or at 12 hours after nephrectomy. The data were analyzed statistically for the effects of these experimental procedures on the anticonvulsant potency and the duration of action of the drug. Carbon tetrachloride-induced liver damage markedly increases both the potency and the duration of action of trimethadione. The most significant increase in potency occurs at the longer time intervals. In contrast, nephrectomy does not significantly alter the anticonvulsant potency or the duration of action. These data suggest that

in rats the liver is the principal site for the degradation of trimethadione to products devoid of anticonvulsant action, and that nephrectomy plays no significant role in the fate or the excretion of this compound. Similar studies are being conducted in order to determine the effects of these same procedures on the anticonvulsant properties of paramethadione. (Research supported by a grant from National Institutes of Health, Public Health Service.)

New alkaloids of veratrum. EDWARD D. SWISS* AND GEORGE L. MAISON. *Dept. of Pharmacology, Boston Univ. of School of Medicine, Boston, Mass.*

Six new ester alkaloids have been recently isolated from the several species of Veratrum by the Department of Organic Chemistry, Riker Laboratories, Los Angeles, California. These alkaloids have been tested for their hypotensive properties according to the method of Maison, Gotz and Stutzman (*J. Pharmacol. & Exper. Therap.* 103: 74, 1951). Dosage of each alkaloid adequate to produce 30% fall of mean arterial pressure in anesthetized dogs was determined by 10-minute infusion with comparison to standard dose of reference powder (Veriloid) in same animal. Doses in μg/kg/minute were: Alkaloid VB (Veratrum Viride) 0.256 (0.21–0.33); Alkaloid VFA (Veratrum fimbriatum) 1.1 (0.95–1.4); Alkaloid VFB (Veratrum fimbriatum) 0.12 (0.11–0.14); Alkaloid VFC (Veratrum fimbriatum) 1.2 (0.9–1.7); Alkaloid VFD (Veratrum fimbriatum) 0.24 (0.19–0.32) and Alkaloid SAA (Veratrum Eschscholtzii)-0.42 (0.36–0.51). VFB M1 and VFB M2, methanolysis products of alkaloid VFB had the following respective potencies: 0.36 (0.27–0.54) and 0.77 (0.46–2.3). The emetic dose 50 values for dogs by vein expressed as μg/kg. are: VB 4.4 (3.3–5.8); VFB 2.8 (1.9–4.1); VFD 8.8 (7.5–10.2) and SAA 10.8 (8.7–13.4). When the values are corrected for differences in potency, they do not differ significantly from other Veratrum derivatives which have been tested (SWISS, E. D. *J. Pharmacol. & Exper. Therap.* In press; 101: 35, 1951). The mouse LD_{50} values (intraperitoneal) as mg/kg. were: VB 0.34 (0.25–0.4); VFB 0.22 (0.16–0.31), VFD 0.66 (0.54–0.81) and SAA 0.175 (0.06–0.5). The following synthetics were tested for their hypotensive properties: penta-acetyl-germine, isorubijervine diacetate, isorubijervine di-methylethylacetate, O-acetyl-N-methyljervine, O-isobutyl-N-methyljervine, germine triphenylurethane, I-jervine diacetate, N-methyl-x-hydrojervine acetate, isojervine triacetate, germine acetonide acetate and germine monoisobutyrate. The equipotent doses of these substances were all greater than 10 μg/kg/minute.

Cardiovascular actions of adenyl compounds II. VIRGINIA L. SYDOW* AND RAYMOND P. AHLQUIST. *Med. College of Georgia, Augusta.*

We have reported (*Federation Proc.* 9: 319, 1950) that 3-adenylic acid, 5-adenylic acid and adenosine were the only active vasodepressor-vasodilator compounds in a varied group of simple degradation products of nucleic acids. The cardiac effects of these 3 compounds have been further investigated. *1) Perfused rabbit heart:* Single doses, 0.5 mg. or more, produced a transient decrease in rate and amplitude. Continuous perfusion with concentrations ranging upward from 10 μg/ml. slowed the heart for the duration of the perfusion but only transiently decreased the amplitude. No significant changes in coronary flow were found. *2) Anesthetized dogs:* The compounds were administered intravenously, intra-aortically and into the left ventricle; the following were recorded; arterial and left ventricular pressures and the ECG (6 leads consecutively or 2 leads simultaneously). The arterial pressure was decreased, due primarily to peripheral vasodilation as previously reported. The principal ECG change was a shift in the frontal plane T vector coincidental with the fall in arterial pressure. In the unatropinized dog the heart rate was increased during the depressor response. In the atropinized dog the heart rate was usually decreased. Studies are now in progress to elucidate the mechanism of this cardiac slowing produced by these adenyl compounds. (Supported in part by an A. M. A. Therapeutic Research Grant).

Urocanic acid as in intermediate in the enzymatic conversion of histidine to glutamic and formic acids. HERBERT TABOR, ALAN H. MEHLER,* OSAMU HAYAISHI* AND JULIUS WHITE.* *Natl. Insts. of Health, Bethesda, Md.*

Extracts of *Pseudomonas fluorescens* adapted to histidine have been shown to convert histidine quantitatively to glutamic and formic acids and ammonia (TABOR AND HAYAISHI. *J. Biol. Chem.*, 1952). Other workers, using liver enzymes, had obtained these compounds only after chemical hydrolysis of the products of histidine degradation. Since their results led to conflicting proposals for the enzymatic mechanism involved, studies with *Pseudomonas* extracts were carried out using isotopically labeled histidine. Using histidine labeled with N^{15} in the α-amino position, it has been established that this nitrogen is converted to ammonia. The nitrogen of the glutamic acid formed was shown to be derived from the γ (ring) nitrogen of histidine by experiments in which this position was labeled with N^{15}. Similar experiments with 2-C^{14} labeled histidine showed the quantitative conversion of this carbon to formic acid. The fate of the nitrogen atoms is consistent

with the suggested pathway through urocanic acid, but not with the mechanism involving primary rupture of the imidazole ring and persistence of the α-amino group. To test the role of urocanic acid, this compound was incubated with the enzyme and C^{14} labeled histidine. The isotope of the histidine metabolized was trapped by dilution with the added urocanic acid. The specific activity of the urocanic acid isolated at the end of the incubation was sufficient to account for essentially all of the histidine degraded during the experiment.

Effect of whole body roentgen radiation on phagocytic function in rabbits. G. V. TAPLIN, J. S. GREVIOR, C. FINNEGAN, M. L. LANIER AND A. DUNN (introduced by THOMAS J. HALEY). *School of Medicine, Univ. of California at Los Angeles.*

Although mid-lethal doses of radiation produce little morphological change in the relatively radioresistant cells of the reticulo-endothelial system, biological evidence indicates that their phagocytic function is depressed. Bacteremia develops during the acute phase of the radiation syndrome and a parallelism exists between it and the total mortality. It has been observed that, after intravenous injection of 1.3 mg/kg. prodigiosin, this colloidal dye is rapidly distributed throughout the organs of the RE system and is not excreted in the urine. Spectrophotometric analysis of extracts of these organs and the blood plasma serves to measure the degree and rate of clearance of the dye particles from the blood and their deposition in the RE system. Pre- and postirradiation studies on several hundred rabbits subjected to 300 to 1200 r acute whole body x-irradiation revealed that doses of 300 r had little effect upon the blood clearance of prodigiosin. However, at 800 r and above there was a depression of the phagocytic function of the cells of the macrophage system during the first 2 weeks. This was followed by a rapid return to normal or above by the end of the 4th week. It was also observed that an inverse relation exists between spleen weight and prodigiosin blood clearance. There is a reciprocal relationship between prodigiosin uptake in the RE organs and the blood clearance during the first postirradiation month. These findings suggest that prodigiosin might be used as a clinical laboratory test for phagocytic function of the macrophage system to assess the degree of injury produced by acute whole body irradiation. (This paper was based on work performed under Contract No. AT-04-1-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.)

In vitro release of CO_2 by N-alkyl oxazolidine-2,4-diones and their protection against Metrazol convulsions. J. D. TAYLOR AND E. L. BERTCHER (introduced by R. K. RICHARDS). *Dept. of Pharmacology, Abbott Labs., North Chicago, Ill.*

Two effective agents against petit mal epilepsy are CO_2 and certain oxazolidinediones. In a previous abstract (*Federation Proc.* 10: 340, 1951) a manometric method for the determination of Tridione in tissues based on the measurement of CO_2 produced by alkali followed by acid was described. With other N-alkyl oxazolidine-2,4-diones it was found that the amount of CO_2 evolved by the above treatment depended on the substituents on the ring. The relative amount of CO_2 produced by an oxazolidinedione in 30 minutes was compared with its intraperitoneal dose giving complete protection against Metrazol-induced convulsions in mice (100 mg/kg. subcutaneously). It was found that the potency of water-soluble, N-alkyl oxazolidine-2,4-diones against Metrazol-induced convulsions was proportional to the relative amount of CO_2 produced *in vitro*. In 7 water-soluble compounds the product of the effective anti-Metrazol dose and the molar fraction of CO_2 liberated *in vitro* was relatively constant. Although this might seem to implicate CO_2 as the common effective agent, it is also possible that the relative rate of formation of the unstable carbonate intermediate may be involved.

Epinephrine and arterenol cutaneous vasoconstriction and adrenergic blockade. JAMES P. TAYLOR* AND RAYMOND P. AHLQUIST. *Med. College of Georgia, Augusta.*

The intracutaneous injection of vasoconstrictors such as epinephrine delays the appearance of the skin blueing response produced by the local application of chloroform in rabbits treated with Trypan Blue. Using this test, the comparative cutaneous vaosconstriction produced by *levo*-epinephrine and *levo*-arterenol, and the effect of adrenergic blockade thereon, have been determined in anesthetized rabbits. Concentrations of epinephrine and arterenol ranging from 1×10^{-4} to 1×10^{-8} molar were compared. Epinephrine was found to be more active than arterenol. Equivalent delays in the skin blueing response were obtained with concentrations of epinephrine $\frac{1}{10}$ those of arterenol. These results are in agreement with the postulated comparative effects of these amines as presented by this laboratory (*Am. J. Physiol.* 153: 586, 1948). Adrenergic blocking agents, including Piperoxan, Regitine, Ro 2-3248, CCK-197, Dibenamine, SY-28 and SKF-688A were administered intravenously at variable intervals before the epinephrine and arterenol. All of the blocking agents diminished, or abolished, the cutaneous vasoconstriction, depending on the dose administered and the time of injection. Ganglionic blocking agents were without signifi-

cant effect. Comparative data will be presented. (Supported by a grant from the American Heart Association.)

Lack of toxicity of parathion spray residue in orange peel and juice. CLINTON H. THIENES, ORSELL M. MEREDITH,* EDMUND PRESCOTT* AND JOHN PARSONS.* *School of Medicine, Univ. Of Southern California, Los Angeles.*

Oranges were sprayed with a suspension of 4 pounds of 25% wettable powder of parathion in 100 gal. water at the University of California Citrus Experiment Station orchard under the direction of Dr. A. M. Boyce; the fruit was picked 2 days later, juiced, and the peel dried and ground under the supervision of W. E. Baier at the By-Products Research Laboratories of the California Fruit Growers Exchange. Groups of 12–20 male, and 12–20 female white rats were fed the following diets: standard powdered laboratory diet, standard diet plus 25% *parathion-sprayed* dried peel, standard diet plus 25% *unsprayed* dried peel, standard diet and equal parts of water and orange juice from *sprayed* and from *unsprayed* fruit, respectively, in the drinking fountain. Half the experiment was with fruit sprayed at the beginning of the experiment and used throughout the 48 weeks of the experiment; the other half was with fruit processed weekly and fed the following week for 48 weeks. As judged by the growth curves, lack of unusual symptoms attributable to parathion, comparative mortality, and cholinesterase activity of the blood, it was apparent that the amount of parathion remaining on the dried peel was not sufficient to cause poisoning. The dried peel contained 4–25 parts/million, the juice no parathion. (Supported by a grant from the American Cyanamid Company.)

Effect of administration of adrenocorticotrophic hormone on acetylcholine content of the brain. CLARA TORDA AND HAROLD G. WOLFF. *Depts. of Medicine (Neurology) and Psychiatry, New York Hosp.-Cornell Med. Center, New York City.*

Administration of the adrenocorticotrophic hormone of the pituitary gland (ACTH) to man and animals induces symptoms suggesting changes in the acetylcholine metabolism. It is known that prolonged administration of ACTH increases the ability of brain to synthesize acetylcholine. Furthermore, prolonged administration of ACTH increases somewhat the activity of acetylcholine esterase. In the following the effect of administration of ACTH on the acetylcholine content of the brain was investigated. The total acetylcholine content of the brain homogenates was assayed biologically on the sensitized rectus abdominis muscle of the frog. Within 2–5 minutes after intraperitoneal administration of ACTH the acetylcholine content of the brain increased. This was followed by a return of the acetylcholine content within normal limits in about 15 minutes after administration of ACTH. The acetylcholine content of the brain approximated normal values in animals receiving ACTH for several days and in hypophysectomized animals.

Survival of rats with high free acetylcholine (ACh) in the brain. W. W. TOURTELLOTTE (introduced by J. M. COON). *Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.*

Sprague-Dawley rats were poisoned with lethal doses of di-isopropyl fluorophosphate (DFP), 4 mg/kg., i.m. 5 minutes after being pretreated with neostigmine methyl-sulfate (N), 0.1 mg/kg., or physostigmine salicylate (P), 0.15 mg/kg., subcutaneously. Rats treated with N-DFP had brain cholinesterase (ChE) activity of 1–2% of the initial activity at 5 hours, whereas P-DFP treated rats had 19–24% of the initial activity at 1 hour, and 8–12% at 8 hours. In rats treated with N-DFP, the free ACh concentration in the brain rose linearly from 0.8 μg/gm. to a maximum of 5.2 at 1.0 hour before decreasing. This is in contrast to P-DFP treated rats in which the free ACh rose linearly to a maximum of 3.8 at 0.5 hour before decreasing. With N-DFP treated rats the free ACh concentration of the submaxillary gland rose from 1 μg/gm. to a maximum of 1.6 at 0.5 hour before decreasing; whereas, with P-DFP treated rats the free ACh concentration rose to a maximum of 3 at 0.5 hour before decreasing. The skeletal muscle free ACh did not change with either treatment. At the time of death, approximately ½ hour, the rats poisoned with DFP alone had similar free ACh concentrations in the brain, in the submaxillary gland, and in the muscle as did those rats treated and surviving P-DFP treatment. The data indicate that high free ACh concentration (6.5 times normal) and low ChE activity (1–2% of normal) in the brain is compatible with life in Sprague-Dawley rats.

Effect and distribution of local anesthetics in the normal and desheathed nerve. A. P. TRUANT AND VINCENT LANZONI (introduced by BYRON B. CLARK). *Pharmacology Dept., Tufts College Med. School, Boston, Mass.*

Attention has been focused on a direct approach for studying the role of the nerve sheath in the diffusion of local anesthetics into the main portion of the normal, desheathed and resheathed bullfrog sciatic-peroneal nerve. The distribution of cocaine, procaine, zylocaine and monocaine was determined quantitatively in the nerve, and results were correlated with their blocking effects on the action potential. In comparison to the normal nerve, the desheathed nerve was found to be 8–10 times more responsive to the blocking action of all agents. Experiments on the perfusion

of the isolated sheath have been undertaken to elucidate this finding. The resheathed nerve was found to behave more like the normal nerve. It was noted that changes in the diameter of the desheathed nerve occurred with time. Using effective blocking concentrations the uptake of drug by the nerve follows a linear function; this is not the case during the release of the drug from the nerve. During the first 60 seconds almost 50% of the total drug concentrations picked up by the normal and desheathed nerve is released. In excess of 75% is liberated by the 5th minute. The remainder is set free over the next 60-minute period. This indicates that the nerve fiber has the ability to retain the drug. Further, the epineurium appears to have no major influence during the release phenomena.

Local anesthetic studies with procaine and xylocaine. PAUL E. TULLAR (introduced by HERBERT A. BRAUN). *Dept. of Pharmacology, Univ. of Texas Dental Branch, Galveston.*

Two series of observations on adult male subjects are cited: *1*) in which tooth pulp sensitivities were measured before and after 'mandibular blocks' with procaine and with xylocaine; and *2*) in which sensitivites of surface areas of the hand were measured before and after subcutaneous infiltrations with these drugs. High frequency currents, adjusted to minimum effective amperages, were applied to vital tooth structures with professional vitality testing instruments in the first series to produce characteristic sensations serving to measure the subjects' algesic state. Skin sensitivities in the second series were measured according to the exposure times needed to produce momentary stings with a focused light ray of pre-established intensity, By using intensities which produced this stinging with approximately 10 seconds' exposure, hyperalgesia as well as hypo-algesia could be measured. Two per cent solutions of procaine hydrochloride and xylocaine hydrochloride were used in each series—without other vasoconstrictors. Approximately 1.5-cc. volumes were used in the mandibular injections; 0.15-cc. volumes were used for the surface infiltrations; and no other surgery was performed. Responses were timed or measured mechanically by a skilled operator, and the subjects were kept in ignorance of the drug being used. Subjective notations were made without prompting or suggestion. Procaine and xylocaine produced effective degrees of nerve block and anesthesia in the concentration and volumes described. Anesthesias were somewhat slower in onset, of shorter duration, and less uniformly complete following procaine; and undesirable hyperalgesias were frequently observed following procaine anesthesias.

Further studies on effect of pentobarbital sodium on adrenalectomized rats. JAMES R. TUREMAN,* ARNOLD H. MALONEY, CLEO FROIX* AND WALTER M. BOOKER. *Dept. of Pharmacology, Howard Univ. Med. School, Washington, D. C.*

Preliminary experiments in this laboratory have shown that the sleeping time of adrenalectomized rats is increased more than 100% beyond that of the intact controls when administered 30 mg/kg. pentobarbital sodium. Furthermore, both female intact and female adrenalectomized animals sleep longer than their counterparts of the male sex. Treatment of the adrenalectomized animals with cortisone, DCA and ascorbic acid, revealed somewhat of a confused picture; ascorbic acid lengthened the sleeping time in both intact and adrenalectomized animals; cortisone lessened somewhat the sleeping time of the adrenalectomized animals, but lengthened that of the intact animals, DCA was more effective in diminishing sleeping time of females than of males. Early experiments have indicated a difference of the 'waking' blood levels of the barbiturate in the intact and adrenalectomized animals. Experiments in progress are designed to extend the preliminary observations.

Action of atropine on sympathetic ganglia. KRISTEN M. TVEDE* AND RAYMOND L. CAHEN. *Maltbie Labs., Morristown, N. J.*

There is a discrepancy of results regarding the effect of atropine on transmission through autonomic ganglia, probably because different experimental conditions and methods were used. The present study concerns several criteria of ganglionic activity investigated at various dose levels of atropine. In only 4 of 7 cats anesthetized with Dial, atropine (1 mg/kg. intravenously) significantly relaxes the sustained contraction of the nictitating membrane elicited by electrical stimulation of the preganglionic nerve fibers; 2 and 3 mg/kg. are effective in 3 other animals. No relaxation is observed after atropine when stimulation is applied to the postganglionic fibers. In 6 cats under chloralose, atropine up to 3 mg/kg. intravenously does not decrease the carotid sinus pressor reflex. In 6 other cats submitted to artificial respiration, 6–10 mg/kg. is required to block the pressor reflex resulting from carotids occlusion. The pressor response to acetylcholine (300 γ/kg.) and 2 ganglionic stimulant agents, 1-dimethyl-4-phenylpiperazinium iodide (40 γ/kg.) and 2-benzylphenyl-B-dimethylamino-ethyl ether methiodide (30 γ/kg.) is not significantly decreased unless a dose from 6–15 mg/kg. is injected. Such ganglionic blocking action, however, is of shorter duration than the anticholinergic one.

Effectiveness of anticurarizing agents at different frequencies of stimulation. E. F. VAN MAANEN. *Pharmacology Dept., Univ. of Cincinnati College of Medicine, Cincinnati, Ohio.*

In anesthetized rats paralysis of the sciatic

nerve-muscle preparation was produced by intravenous injection of *d*-tubocurarine. The rates of stimulation studied were 5, 2, 0.5, and 0.2 stimuli/second. Increase in frequency of stimulation causes a decrease of the paralyzing $dose_{50}$ (PD_{50}) of *d*-tubocurarine (*J. Pharmacol. & Exper. Therap.* 103: 358, 1951). In order to test the anti-curare effect of tensilon chloride (Ro 2-3198) and neostigmine methylsulfate it is desirable to use doses which produce maximal effect but do not of themselves depress the twitch height. In the case of tensilon the range of doses which produces maximal anticurare effect is narrow (1–3 mg/kg. at 0.5 stimuli/second). With 1 mg/kg. the anti-curare effect decreases at the highest rate of stimulation. But at this requency the PD_{50} of tensilon itself is 3.8 mg/kg. Neostigmine has a wider range of doses which produces maximal anticurare effect (0.03–0.3 mg/kg. at 0.5 stimuli/second) and the dose of 0.03 mg/kg. is no greater that $\frac{1}{50}$ of the depressant dose even at the highest frequency used. The relative anti-curare effect of this dose of neostigmine is the same at all 4 frequencies, i.e. the ratios of the PD_{50}'s after neostigmine over their control values are, respectively, 2.03, 1.93, 1.98, and 2.02.

Effect of drugs on tremor in monkeys (motion picture). VERNON G. VERNIER (introduced by KLAUS R. UNNA). *Dept. of Pharmacology, Univ. of Illinois College of Medicine, Chicago.*

Tremor at rest in monkeys has been produced by the method of Ward and Peterson by placing small bilateral electrolytic lesions in the midbrain of monkeys (*Macaca mulatta*). Using the Horsley-Clarke stereotaxic instrument these lesions were located in the reticular formation of the midbrain between the substantia nigra and the red nulceus and were about 2 mm. in diameter. The positions of the lesions were verified histologically and are illustrated. Following recovery, these monkeys manifested postural tremor in the extremities at a rate of about 6–8/second, fairly closely resembling the tremor of human parkinsonism. Neurological examinations revealed normal patellar reflexes and unimpaired ability to grasp. The tremor disappeared with voluntary activity of the involved extremity. The motion picture shows the inhibition of tremor for over an hour after apomorphine (0.1 mg/kg., intramuscularly). The tremor gradually returns so that 80 minutes after the injection it reaches the control amplitude and strength. Both the normal control and tremor monkeys display considerable excitement with this drug. The film also includes illustrations of suppression of hyperkinesia by apomorphine as demonstrated in the electromyogram of a tremor monkey. Similar results are shown in a tremor recording from a Parkinsonian patient. (Supported by a research grant from the National Institutes of Health, Public Health Service.)

Enzymatic mechanism of creatine synthesis. PAUL J. VIGNOS, JR.* AND G. L. CANTONI. *Dept. of Pharmacology, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

Creatine synthesis in liver homogenates involves methylation of guanidoacetate. L-Methionine is the methyl donor compound; the reaction requires adenosinetriphosphate and Mg^{++} (BORSOOK AND DUBNOFF. *J. Biol. Chem.* 171: 363, 1947; COHEN. *J. Biol. Chem.* 193: 851, 1951). Previous work on an analogous transmethylation reaction, the biosynthesis of N′-methylnicotinamide, led to the suggestion that activation of methionine is a prerequisite for the transfer of its methyl group (CANTONI. *J. Biol. Chem.* 189: 745, 1951). It has now become clear that activation of methionine by *reaction 1* (L-methionine + ATP $\xrightarrow{GSH,\ Mg^{++}}$ 'active methionine' + inorganic phosphate) can be coupled with *reaction 2* (active methionine + guanidoacetate → creatine + X) to give creatine. An enzyme catalyzing *reaction 2* has been found in extracts of guinea pig, rabbit, beef and pig liver. Pig liver extracts have been purified 15 to 20-fold by ammonium sulfate fractionation followed by adsorption on and elution from alumina C_γ. The partially purified enzyme, guanidoacetate methylpherase, is free of methionine-activating enzyme. It will not methylate nicotinamide. Oxygen, SH-groups or Mg^{++} are not required for its activity. One of the reaction products has been identified as creatine by: *a*) the α-naphthol-diacetyl creatine reaction; *b*) conversion to creatinine determined as the alkaline picrate following adsorption on Lloyd's reagent. The nature of other reaction products is under investigation.

An improved method for the determination of 5-iodo, 1-thiouracil (Itrumil) in blood. GILMOUR WAGLE* AND A. J. PLUMMER. *Research Labs., Ciba Pharmaceutical Products, Inc., Summit, N. J.*

Sodium-5-iodo, 2-thiouracil (Itrumil), as reported previously, can be extracted from blood plasma with butyl acetate after acidifying with hydrochloric acid. Itrumil is removed from the butyl acetate with sodium hydroxide and this extract, after acidification with acetic acid, is applied to a sheet of Whatman No. 1 filter paper. The chromatogram is developed for 16 hours in a butanol-water system according to the technique of Shabica and Solook (*Federation Proc.* 9: 314, 1950). After drying in the hood at room temperature the chromatogram is examined under ultraviolet light. An absorption band is identified at R_f 0.7 and its outline clearly defined with pencil. The intensity of this band gives an indication as to the amount of Itrumil present. The outlined area of the paper is cut out and is twice shaken for an hour with 2 10-cc. portions of methyl alcohol.

The paper is finally washed with an additional 5 ml. of methyl alcohol. The combined methyl alcohol eluates are allowed to evaporate in a hood to dryness. The residue is dissolved in 3.0 ml. of distilled water; 0.6 ml. of a pH 7.0 buffer is added. After mixing, 0.1 ml. of Grote's reagent is added to develop a green color. The absorption is read at 720 μ after 5 minutes, using the Beckman Spectrophotometer. Determination of the Itrumil present is based on a calibration curve prepared by the determination (in the above manner) of known amounts of Itrumil added to plasma.

Emetic trigger zone and motion sickness in dogs. S. C. WANG* AND H. I. CHINN. *College of Physicians and Surgeons, Columbia Univ., New York, and School of Aviation Medicine, Randolph Field, Tex.*

Wang and Borison (1950) have localized a chemoreceptive trigger zone for emesis in the caudal portion of the medulla. Ablation of this zone protects dogs from vomiting to apomorphine and other centrally acting emetics. The vomiting center remains intact in these animals, because they vomit to threshold doses of orally administered copper sulphate. Twelve dogs which have been consistently sensitive to swinging motion on 4 successive weekly trials are selected for the operations: bilateral labyrinthectomy (3 dogs), extirpation of nodulus and uvula (3), trigger zone ablation (5) and sham operation (one, which showed no change in sensitivity in motion sickness). The results shown in the labyrinthectomized and cerebellar animals are in conformity with those reported by Sjoberg (1931) and by Tyler and Bard (1949), respectively. The findings on trigger zone ablated dogs (see table) indicate that the chemoreceptive zone is in the direct pathway concerned with the vestibular reflex in producing motion sickness. The mechanism involved in the mediation of impulses to this zone is now under study.

OPERATION—TRIGGER ZONE no.	POSTOPERATIVE THRESHOLDS Apomorphine mg.	Copper sulphate mg.	ROTATION TESTS (Nystagmus)	SWING TESTS (Vomit)
7	5.0(?)	40	Positive	Occasional delayed positive
8	10.0	40	Positive	Negative
9	5.0	40	Positive	Negative
10	10.0	160	Positive	Negative
11	0.2	160	Positive	Negative

Effect of adrenergic blocking agents on ether hyperglycemia in rabbits. D. T. WATTS. *Dept. of Pharmacology, Univ. of Virginia Med. School, Charlottesville.*

There is considerable evidence that ether at anesthetic levels produces a hyperglycemia by stimulating the sympathico-adrenal system with the subsequent release of epinephrine and mobilization of liver glycogen. In the present investigation Dibenamine, Priscoline and Hydergine (a mixture of equal parts of the methanesulfonates of dihydroergocornine, dihydroergocristine and dihydroergokryptine) have been used in an attempt to block this hyperglycemic action of ether. Each drug was tested on a group of 12 rabbits and the values given below are the means for each group. Thirty minute surgical anesthesia, as determined by the absence of the corneal reflex, elicits a significant hyperglycemia in well-fed rabbits and has been used in all experiments. In an untreated group of rabbits the blood glucose increased 22.3 mg. %. This increase is highly significant; $P > 0.01$. When 0.15 mg/kg. of Hydergine was given 30 minutes before anesthesia the increase in blood glucose was only 2.6 mg. %. This increase is not significant; $P > 0.5$. After a dose of 5.0 mg/kg. of Priscoline administered 30 minutes before anesthesia the blood sugar level rose 16.0 mg. %. This is a significant increase, $P > 0.02$. After a 50 mg/kg. dose of Dibenamine given 20 hours before ether anesthesia the increase in blood glucose was 24.9 mg. %. This is a significant increase; $P > 0.01$. These results show that Hydergine is a very effective inhibitor of ether hyperglycemia in rabbits whereas Dibenamine and Priscoline at dosages approaching toxic levels have no effect on the hyperglycemia produced by ether anesthesia in rabbits. This is considered as indirect evidence that the adrenergic blocking action of Hydergine is different from that of Dibenamine and Priscoline.

Further development of a mechanical heart-lung for the study of drugs on the peripheral circulation. RUSSELL A. WAUD. *Dept. of Pharmacology, Med. School. Univ. of Western Ontario, London, Canada.*

Progress in the development of a mechanical heart and lung will be described. Graduated glass syringes are used as pumps. The stroke output may be read at any moment or varied at will. By means of a special cam the changes in volume of the syringes are made to follow the volume curve of the mammalian ventricle and the vascular system to pulsate physiologically. The artificail lung consists of a glass percolator in which oxygenation of the blood depends mainly on passing the blood through an aggregation of bubbles rather than the oxygen through the blood. By means of special cannulae a technique has been worked out whereby the time required to transfer from the animal's heart and lungs to the mechanical heart and lung is reduced to 10–20 seconds; thus the nervous system is subjected to very little hypoxia. Ordinary

eye and other reflexes are present for hours. Its use in pharmacological studies will be discussed.

Tissue distribution and excretion of *d*- and of *l*-methadone. E. LEONG WAY AND CHEN-YÜ SUNG.* *College of Pharmacy and Div. of Pharmacology and Exptl. Therapeutics, School of Medicine, Univ. of California, San Francisco.*

Although *l*-methadone and *d*-methadone differ markedly in their pharmacologic actions, especially on the central nervous system, little is known about the metabolic fate of these two optical isomers. Consequently, a study on the fate of *d*- and *l*-methadone was initiated in rats, the determination of these compounds in tissues and excreta being carried out as previously reported for *d,l* methadone (WAY, E. LEONG., C-Y. SUNG AND W. P. MCKELWAY, *J. Pharmacol.* 97: 222, 1949). The distribution of these isomers in the various tissues studied followed the same pattern as that of racemic methadone, with high concentrations being found in the lungs, liver, kidney and spleen and low concentrations in blood, brain, muscle and heart. The tissue levels of each of the isomers and of the racemic mixture are essentially of the same order of magnitude. Despite the greater analgetic potency of *l*-methadone, it is not localized to any higher degree by the brain than the *d*-isomer. Only a minor fraction of each isomer is excreted in the urine and feces. Counter-current studies on rat urine indicate that the *d*-isomer may be metabolized to a slightly greater extent than the *l*-isomer. The results to date reveal no clue to explain the difference in pharmacologic actions of the optical isomers of methadone. (Supported in part by the Natl. Insts. of Health, Bethesda, Md.)

Potency of antiepileptic drug combinations: phenobarbital and diphenylhydantoin on maximal electroshock seizures. LAWRENCE C. WEAVER,* EWART A. SWINYARD, LOWELL A. WOODBURY AND LOUIS S. GOODMAN. *Depts. of Pharmacology and Physiology, College of Pharmacy and College of Medicine, Univ. of Utah, Salt Lake City.*

The ED_{50}'s of phenobarbital, diphenylhydantoin and combinations of these drugs were determined in rats; the end point employed was abolition of the tonic extensor component of maximal electroshock seizures. The drugs were combined in various ratios on the basis of anticonvulsant effectiveness: the ED_x of one drug was merged with the $ED_{50} - ED_x$ of the other drug and the resulting mixture tested for its ED_{50}, 2 hours after subcutaneous administration, as if it were a new drug. Results were analyzed statistically by a modification of the method for drug combinations given by Finney (*Probit analysis*, chap. 8, Cambridge, 1947) in order to determine whether potentiation, simple addition or antagonism of drug effects was evident. Potentiation occurred over a very narrow dose range. It was greatest with the $ED_{97.5}$ of phenobarbital plus the $ED_{50} - ED_{97.5}$ of diphenylhydantoin. This combination is approximately 1.7 times more potent than would be expected if the effects were simply additive. There are no significant differences from the calculated ED_{50}'s when ED_x ($x \geqslant 25$) of diphenylhydantoin is combined with the $ED_{50} = ED_x$ of phenobarbital; however, there is a slight tendency toward antagonism. The potencies of the above combinations, using the minimal electroshock seizure, hyponatremic and Metrazol tests, are under investigation. In addition, these studies are being extended to other combinations of therapeutically useful antiepileptic drugs. (Research supported by grant from Natl. Insts. of Health, Public Health Service.)

Relationship of potassium to effects of phenobarbital on QO_2 of brain slices. B. A. WESTFALL. *Dept. of Physiology and Pharmacology, Univ. of Missouri School of Mediine, Columbia, Mo.*

As calcium and magnesium have been shown to be essential for an increase in the Q_{O_2} of cerebral cortex slices after the addition of small concentrations of phenobarbital, it seemed desirable to determine whether or not potassium is necessary for such an increase in Q_{O_2}. The Q_{O_2} of cerebral cortex slices from each of 10 rats was determined by the Warburg technique (37° C.) using Kreb's Ringer-phosphate buffer solution carrying 0.2 % glucose. Slices from each animal were placed in 3 substrates: *1*) without potassium, *2*) with .0064 M potassium and *3*) .0128 M potassium. Phenobarbital tipped in (0.2 mg. %) after a 30-minute control period produced a rise in Q_{O_2} of 10.5 % ($P < .002$) with no potassium, 7.1 % ($P < .006$) with .0064 M potassium and 8.7 % ($P < .001$) with .0128 M potassium in the substrate. Therefore, potassium seems to be unrelated to the increased Q_{O_2} due to the phenobarbital. An additional observation was a higher Q_{O_2} with potassium in the substrate than without the potassium, both in the control period and after addition of phenobarbital.

In vitro studies of renal secretion. VIRGIL D. WIEBELHAUS,* ROBERT L. KEMP* AND KARL H. BEYER. *Pharmacology Section, Research Div., Sharp & Dohme, Inc., West Point, Pa.*

The *in vitro* secretion of phenol red (PSP) and *p*-aminohippurate (PAH) can be inhibited completly by carinamide or 'Benemid' at levels that do not inhibit oxidation or transphosphorylation. Acetate (.01 M) antagonizes the action of these inhibitors. PSP, penicillin and PAH compete for the secretory mechanism *in vitro*. A series of carinamide and 'Benemid' type compounds were tested on *a*) *in vitro* secretion of PSP, *b*) the *in*

vitro secretion of PAH, and *c*) the *in vitro* conjugation of PAB with glycine. Each compound had a similar degree of inhibitory activity in all 3 tests, i.e. there was a definite parallelism which suggests that a conjugation reaction may be an essential step in the renal transfer system. Since Chantrenne demonstrated the CoA requirement of the PAB-glycine conjugase we experimented with renal slices from rats on a pantothenic acid deficient diet. The renal and hepatic tissues of these rats contained only about 50 % as much CoA as the controls. However, renal slices from these rats still secreted PSP and PAH very actively and also gave very good responses to added acetate. If CoA is required for the secretion of these compounds the degree of depletion we attained was not sufficient to demonstrate it. Fluoroacetate inhibits the *in vitro* secretion of PAH, but only moderately decreases the stimulation given by added acetate.

Effects of morphine and N-allylnormorphine on reflexes in dog and cat. ABRAHAM WIKLER AND R. L. CARTER.* *NIMH Addiction Research Center, USPHS Hosp., Lexington, Ky.*

In 18 chronic spinal dogs. morphine (2–100 mg/kg.) depressed flexor and crossed extensor reflexes markedly; the effects of N-allylnormorphine (0.2–100 mg/kg.) were similar but much less intense. Knee jerks were affected variably and slightly by both drugs. Extensor thrusts were unaffected by either drug, enhanced by morphine, or depressed by N-allylnormorphine. N-allylnormorphine antagnoized the depressant effects of morphine on the flexor and crossed extensor reflexes. If N-allylnormorphine was given first, the subsequent depressant actions of morphine were usually blocked (interval between subcutaneous injections 45 ± 15 minutes). When 30 mg/kg. of N-allylnormorphine was injected subcutaneously 1 hour after 100 mg/kg. of morphine, a typical 'morphine abstinence syndrome' appeared both in the segments rostral to the level of transection, and in the paralyzed hindlimbs (5 experiments). Such abstinence syndromes were also observed within 20 minutes after subcutaneous injection of single doses of N-allylnormorphine in 3 'addicted' preparations. Two mg/kg. of the drug produced these effects after morphine had been administered regularly in doses of 2 mg/kg. q6h for 4 days. As addiction continued at the same morphine dose level, the abstinence syndromes which were precipitated by the same single dose of N-allylnormorphine increased markedly. In intact cats, 5 mg/kg. of N-allylnormorphine produced no notable changes in behavior but antagonized the 'motor excitant' effects of 5 mg/kg. of morphine. In acute spinal cats, N-allylnormorphine, 0.5–15 mg/kg., enhanced the flexor and crossed extensor reflexes, blocked depressant effects of morphine thereon, or reversed them if morphine was injected first.

Precipitation of 'abstinence syndromes' by single doses of N-allylnormorphine in addicts (motion picture). ABRAHAM WIKLER, R. L. CARTER,* H. F. FRASER* AND HARRIS ISBELL. *NIMH Addiction Research Center, USPHS Hosp., Lexington, Ky.*

During active addiction to morphine or methadone, acute 'abstinence syndromes' appeared after subcutaneous injection of single doses of N-allylnormorphine. In both morphine and methadone addicts, the syndrome resembled strikingly that which is observed after abrupt withdrawal of morphine, except that after N-allylnormorphine, withdrawal signs appeared within 15 minutes, reached peak intensity by 45 minutes and then subsided slowly. One hour after N-allylnormorphine, even intravenous injection of morphine or methadone had little ameliorating effect on the 'abstinence syndrome'. Such abstinence syndromes could be demonstrated by subcutaneous injection of single doses of 15 mg. of N-allylnormorphine as early as one week after experimental addiction to morphine 15 mg. or methadone 10 mg. four times daily. After stabilization on morphine (360 mg. per day) or methadone (140 mg. per day), severe abstinence syndromes were precipitated by 5 mg. of N-allylnormorphine. The most intense abstinence syndrome ever observed in this institution occurred when 30 mg. of N-allylnormorphine were administered subcutaneously to a subject who had been stabilized on 300 mg. of morphine per day. In contrast, definite withdrawal signs were precipitated by 15 mg. of N-allylnormorphine in meperidine addicts only when the total daily dose of meperidine was about 3000 mg. Further studies on the effects of N-allylnormorphine in the course of addiction to other narcotics are in progress.

Effects of urethane and carbon tetrachloride on alkaline phosphatase (APH) activity of mouse livers. W. LANE WILLIAMS, RICHARD B. ARONSOHN AND RICHARD H. MEYER (introduced by R. N. BIETER). *Dept. of Anatomy, Univ. of Minnesota, Minneapolis.*

Following 4–16 hours in substrate (glycerophosphate, modified Gomori) APH activity is demonstrated in hepatic parenchymal cytoplasm of mice receiving urethane or carbon tetrachloride, but not in livers of normal mice, or those similarly injected with sodium barbital or trional (*Federation Proc.* 10: 346, 1951). Nuclei showed APH activity in all of these mice. Due to criticism of methods commonly used for APH localization (NOVIKOFF. *Science* 113: 320, 1951; GOMORI. *J. Lab. & Clin. Med.* 37: 526, 1951) these studies were

repeated and extended. Decrease of incubation-time to 1–3 hours and control of *ph* (9.2) showed in normal livers a limitation of activity to biliary channels; or with maximum incubation, an additional faint nucleolar and nuclear membrane activity. Livers of urethane-injected mice showed strong APH activity in parenchymal cytoplasm, increased activity in biliary channels, and stronger nuclear reactions without histological evidence of injury. Following injection of carbon tetrachloride, damaged centrilobular cells showed intense cytoplasmic APH activity with little significant activity in extracentral cells. Although both extended and brief periods of incubation show differences between livers of normal and those of urethane or carbon tetrachloride-injected mice, the shorter incubation-time produces a more accurate picture of APH activity in that false reactions resulting from absorption are limited or eliminated.

Loss of pharmacologic effects of x-rayed epinephrine solutions. JAMES E. WILSON,* L. E. ELLINWOOD* AND J. M. COON. *U. S. Air Force Radiation Lab. and Dept. of Pharmacology, Univ. of Chicago, Chicago, Ill.*

The injection of a 1:100,000 dilution of epinephrine which had been exposed to 250 kv. x-ray doses of 25,000 to 100,000 r into the perfused isolated rabbit heart produced a brief slowing of the rate without change in amplitude. Such irradiated solutions also showed a diminished inhibitory action on isolated rabbit intestine and a decreased pressor activity in the anesthetized dog. A commercial solution of epinephrine hydrochloride, containing sodium bisulfite as a preservative, lost 63% and 80% of its pressor activity after doses of 50,000 and 100,000 r, respectively. A solution made by dissolving crystalline epinephrine bitartrate in 0.9% saline showed losses in activity of 72 and 88%. Both preparations failed to elicit any pressor response when the x-ray dose was increased to 200,000 r. Less pronounced losses of pressor activity were obtained in more concentrated solutions. Assays performed by a fluorophotometric method, which involved the oxidation of epinephrine to adrenochrome and further rearrangement to adrenolutine, gave results that paralleled the loss of pressor activity. A similar destruction of *nor*-epinephrine by x-rays was observed when 1:100,000 dilutions were tested for pressor activity and assayed fluorophotometrically. The exposure of ephedrine in a concentration of 3 mg/ml. to 100,000 r resulted in no demonstrable change of pressor activity. That adrenochrome is not the end product of the action of x-rays on solutions of epinephrine is evident by its failure to be detected spectrophotometrically and by the absence of a pink discoloration. Studies on the rate of destruction of epinephrine in various concentrations by x-rays have been carried out.

Hemodynamic factors in the control of coronary circulation in the dog. MARTIN M. WINBURY AND LYLE D. CALVIN (introduced by W. E. HAMBOURGER). *Div. of Biological Research, G. D. Searle and Co., Chicago, Ill.*

This investigation was undertaken to determine the relative importance and interrelationship of the various hemodynamic factors involved in the regulation of the coronary blood flow rate (CBF). To allow the coronary arterial pressure to vary independently of the systemic arterial pressure, the left coronary artery was perfused with blood from a donor dog by cross circulation. The effects of variation in coronary pressure, systemic pressure, cardiac output, cardiac work, cycle length, systolic/diastolic ratio and coronary resistance on CBF were studied when the coronary pressure and/or systemic pressure were altered experimentally. The data were analyzed by multiple and partial correlation methods. Analysis indicates that the major portion of the variation in CBF in the anesthetized dog can be explained by the variation in the 3 factors, coronary arterial pressure, systolic/diastolic ratio and coronary resistance. Under sympathetic stimulation, CBF is increased independently of any change in coronary arterial pressure; the systolic/diastolic ratio may either increase or decrease while the coronary resistance declines.

Production of controlled neuromuscular block by infusion of diacetylcholine. A. LLOYD WNUCK,* STATA NORTON,* C. H. ELLIS* AND E. J. DE BEER. *Wellcome Research Labs., Tuckahoe, N. Y.*

The neuromuscular block produced by diacetylcholine (succinylcholine) is known to be of short duration. The rapid development of marked paralysis and the rapid recovery from the paralysis has suggested that continuous infusion of diacetylcholine might give a greater control over the depth and duration of neuromuscular paralysis than is possible with longer acting 'curariform' drugs. Infusions of diacetylcholine at sufficient rates and concentrations to maintain controlled depths of neuromuscular paralysis lasting from 10 minutes to 4 hours have been performed in both cats and dogs. Infusions producing 10–100% block of the gastrocnemius-sciatic preparation have been tried. Recovery from the neuromuscular paralysis is prompt, and shows little difference whether the paralysis was of short or long duration. Where sufficient diacetylcholine was given to block respiration, recovery of spontaneous respiration generally was also quite prompt. The degree of respiratory involvement was comparable to that produced by other 'curariform' agents. Other experiments in which repeated doses of

diacetylcholine were given instead of continuous infusion, an interval for recovery having been allowed to elapse between doses, have shown that there is only slight cumulative action. In the dosage used for the infusion studies, few if any side reactions were encountered in either anesthetized or unanesthetized animals.

Some pharmacological effects of allethrin. GEOFFREY WOODARD, WILLIAM D. HARKNESS AND STANLEY E. SRENSEK (introduced by ARNOLD J. LEHMAN). *Div. of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington, D. C.*

Although allethrin, a synthetic analogue of pyrethrin, is becoming widely used in aerosols and other insecticidal formulations with consequent direct human exposure, little has appeared on its pharmacology. Unanesthetized rabbits given 5 mg. of allethrin/kg. intravenously exhibited generalizea muscular twitching, occasional convulsions, and increased sensitivity to stimuli. These symptoms lasted for 1–2 hours and were blocked by ether and chloroform, essentially unaffected by atropine and increased by physostigmine and picrotoxin. Injection of allethrin in phenobarbital anesthetized dogs and rabbits produced little change in blood pressure but stimulated respiration. Normal rhythm of isolated rabbit intestine was reversibly inhibited by 8 μg. allethrin/ml. of bathing fluid. During inhibition the intestine was insensitive to nicotine and acetylcholine, but reacted to barium chloride. The response of the isolated rat diaphragm to standard phrenic nerve stimuli was first increased, then reversibly blocked by 10 μg. allethrin/ml. of bathing fluid. The spontaneously contracting guinea pig uterus was variably affected, both stimulation and depression being observed.

Influence of thyroidectomy, propylthiouracil and thyroxin on brain excitability. D. M. WOODBURY, R. E. HURLEY,* N. G. LEWIS,* M. W. MCARTHUR,* W. W. COPELAND,* J. F. KIRSCHVINK* AND LOUIS S. GOODMAN. *Dept. of Pharmacology, Univ. of Utah College of Medicine, Salt Lake City.*

Two experiments consisting of 4 groups of 8 rats each were performed. The groups were treated as follows: *1*) untreated controls; *2*) propylthiouracil, 2% in food; *3*) thyroidectomized; *4*) thyroxin, 100 μg/day. In *experiment 1*, electroshock seizure thresholds (EST) were determined for 35 days. The following changes in EST were noted: untreated controls, +3.4%; propylthiouracil, +11.4%; thyroidectomized, +7.3%; thyroxin-treated, −10%. In *experiment 2*, the animals were tested for alteration of pattern of maximal electroshock seizure (M.E.S.), recovery time (RT_{50} after M.E.S., and convulsive dose of metrazol (CD_{50}). Propylthiouracil increased flexor and decreased extensor components and increased duration of M.E.S.; thyroidectomy decreased flexor and increased extensor components and increased duration of M.E.S.; thyroxin decreased all 3 indices. The table summarizes effects on metrazol CD_{50} and on RT_{50}. (Figures in parentheses, 95% confidence limits.)

TREATMENT	CD_{50} (MG/KG.)	RT_{50} (MIN.)
Controls...	43.8(38.5–49.8)	8.8 (7.75–10.0)
Propylthiouracil....	56.0(50.1–62.6)	13.3 (12.2–14.4)
Thyroidectomy....	51.9(47.2–57.6)	2.55(1.82–3.57)
Thyroxin..	37.0(33.3–41.1)	7.4 (6.4 –8.6)

Hypothyroidism decreases and hyperthyroidism increases brain excitability. The reason for the opposite effects of thyroidectomy and propylthiouracil on RT_{50} and on flexor and extensor components of M.E.S. pattern is not known. A direct effect of propylthiouracil on the brain is postulated. (Research supported by grant from National Institutes of Health, Public Health Service.)

Effect of Dibenzyline (SKF 688A) on peripheral blood flow in man. D. J. WOODWARD,* S. W. HOOBLER* AND MARK NICKERSON. *Depts. of Medicine and Pharmacology, Univ. of Michigan School of Medicine, Ann Arbor.*

The effects of intravenous Dibenzyline (1.0 mg/kg. in 1 hour), tetraethylammonium (300–400 mg.) or regional nerve block on peripheral blood flow were measured with a venous occlusion plethysmograph in 10 hypertensive patients. Increase in foot blood flow after Dibenzyline averaged 6.5 cc/100 cc/min. (98 ± 19% of the increase noted after nerve block). Tetraethylammonium produced a definitely smaller increase in flow, equal to 46% of the nerve block response. Blood flow reached a maximum ½ hour after the Dibenzyline infusion. Half of this increase persisted several hours. The correlation between the response to Dibenzyline and to nerve block was +0.83. More limited observations indicate comparable increases in hand and forearm blood flow. Toe-thigh and finger-arm temperature gradients were usually abolished, but this response was not well correlated with changes in blood flow. Dibenzyline caused a slight increase in flow in 1 incompletely sympathectomized extremity in which tetraethylammonium and nerve block caused comparable increases, and no significant change in 2 others. A mean fall in blood pressure of 38/25 occurred ½ to 1 hour after the infusion and persisted for 1 to 2 hours. Pulse rate increased an average of 20/ minutes. Dibenzyline infusion caused varying degrees of nasal congestion, orthostatic hypo-

tension and drowsiness. It appears to be an effective, long-acting and well-tolerated peripheral vasodilator, apparently acting through the inhibition of sympathetic vasoconstriction. (Supported by grants from the National Institutes of Health, USPHS and Smith, Kline and French Laboratories).

Akee toxicity. MARK F. WYNN,* EDWARD LARSON AND DONALD D. DOUGHTY.* *Dept. of Physiology and Pharmacology, Univ. of Miami Med. Research Unit at Veterans Administration Hosp., Coral Gables, Fla.*

The akee is a tropical fruit being introduced into Southern Florida. Reports in the literature concerning its toxicity and edibility are conflicting (ARNOLD, L. E. *Jamaica Med. Rev.* 1: 26, 1947). Preparations of the arils or cotyledons of both ripe and unripe fruit have been administered to various laboratory animals. The arils (the edible portion) of the ripe fruit are nontoxic. The arils of the unripe fruit and the cotyledons of both ripe and unripe fruit have shown toxicity. In both dogs and rabbits, the red and white cell counts of the blood are decreased. There is a tendency for the blood chlorides to increase. The toxic principle or principles are heat stable and water soluble, and are not precipitated by the addition of ethyl alcohol. In rabbits there is a declining body temperature, followed by depression and death. At autopsy, the peritoneal cavity is usually very moist, the lungs are congested and the auricles of the heart are distended with venous blood. The small intestine is usually hyperemic and this may also be true of the stomach and large intestine. The kidneys are usually very pale. In some animals there has been discernable hemorrhage in the subcutaneous tissues especially in the area of the external mammary blood vessels.

Antagonistic effect of N-allyl-normorphine on morphine induced hyperglycemia. HOWARD L. ZAUDER (introduced by N. B. DREYER). *Dept. of Pharmacology, Univ. of Vermont College of Medicine, Burlington.*

Intravenous injection of morphine sulphate (2.5–5 mg/kg.) into rabbits results in a rise in blood sugar of 60–120 mg %. Comparable doses of N-allyl-normorphine have no effect on the blood sugar level. Injection of N-allyl-normorphine 5–15 minutes prior to the administration of morphine abolished the early hyperglycemic action of the latter, a slight rise in blood sugar being observed 2 hours after the morphine was introduced. If injection of N-allyl-normorphine followed administration of morphine an initial rise in blood sugar was observed followed by a rapid return to pre-injection levels.

Effects of intraarticular cortisone and hydrocortisone in rheumatoid arthritis. M. ZIFF, E. SCULL AND J. J. BUNIM (introduced by A. C. DEGRAFF). *Dept. of Medicine, New York Univ. College of Medicine, New York City.*

Cortisone and hydrocortisone were injected intraarticularly in 10 patients with rheumatoid arthritis. Seven had bilateral effusions in the knees making possible control observations with the suspending vehicle in the contralateral joint. Five were receiving cortisone systemically in stable dosage during the period of study. Clinical response and changes in properties of the synovial fluid were studied, i.e. cell count, viscosity, A/G ratio, and aminotripeptidase content. The concentration of the latter enzyme in synovial fluid, using glycylglycyl glycine as substrate, has proved a useful index of synovial inflammation. Hydrocortisone in 25-mg. dosage, was effective in suppressing joint inflammation. Cortisone was much less often effective. Eighty-three % of injections of hydrocortisone were followed by at least 2 plus objective improvement in joint status and by significant improvement in the properties of the synovial fluid. After 2 injections, synovial fluid properties were improved in 100% of trials, as based on significant fall in cell count and aminotripeptidase level and rise in viscosity in comparison with the changes in the control joint. Results with cortisone were little better than observed after injection of vehicle. Improvement after hydrocortisone usually began within a day. Though periods of improvement lasting 27–90 days were observed in 3 patients, the average duration in the other patients was 7 days, and the range, 4–15 days. Intraarticular therapy with hydrocortisone is effective, and is especially indicated in the patient with relatively isolated involvement of large joints.

AMERICAN SOCIETY FOR EXPERIMENTAL PATHOLOGY

Thirty-seventh Annual Meeting

NEW YORK CITY, APRIL 14–18, 1952

(An asterisk following an author's name indicates "by invitation")*

Microscopic study of tissue reactions to autologous and homologous musculo-fascial transplants in rabbits. RICHARD H. ANDRESEN,* CLARENCE W. MONROE* AND GEORGE M. HASS. *Rush Lab. of Pathology and Surgery, Presbyterian Hosp., in affiliation with Univ. of Illinois, Chicago.*

Simultaneous autologous and homologous musculo-fascial transplants were made in rabbits. Microscopic studies of the transplants at intervals thereafter showed that the initial pattern of degeneraion and granulation tissue organization of both types of transplant was identical. The superposition of 2 reactions in organizing granulation tissue served to distinguish homologous from autologous transplants. One reaction to homologous transplants was a lymphocytic infiltration and the other an angeitis. The principal locus of these reactions was in the musculo-fascial zone of the transplant and from this zone the reactions spread variably through the fascia into overlying granulation tissue. In single homologous transplants the lymphocytic and mild angeitic reaction predominated. When multiple successive homologous transplants were made from the same donor to the same recipient, acute angeitis with thrombosis supervened and the lymphocytic reaction failed to develop or persist. Multiple successive autologous transplants did not modify the reaction, characteristic of single autologous transplants. Autologous transplants had no influence on the reaction to homologous transplants and homologous transplants did not change the nature of the reaction to autologous transplants.

Chemical studies of ATP-induced contraction of isolated skeletal myofibrils. CHARLES A. ASHLEY,* ALBERTAS ARASIMAVICIUS* AND GEORGE M. HASS. *Rush Lab. of Pathology, Presbyterian Hosp., in affiliation with Univ. of Illinois, Chicago.*

Skeletal myofibrils, isolated from rabbit thigh muscle with a colloid mill and suspended in 0.15M KCl, were purified by differential centrifugation at 0° C. The myofibrils were then washed repeatedly with potassium phosphate-citric acid buffer solution (pH 8, μ 0.154). The concentration of protein in the supernatant of the suspension was determined with the Folin reagent. Washing was continued until the protein concentration could not be determined by this method. The ultra violet absorption curve of the supernatant was too low to be determined. The fibrils were then washed and suspended in 0.15 M KCl containing succinate buffer solution (pH 8.0; ratio, 9:1). These fibrils contracted instantaneously on the addition of ATP. The degree of contraction was proportional to the amount of ATP added. Analyses, using Kalckar's enzymatic and spectrophotometric methods for adenylic acid, ADP and ATP, were made on the ATP stock solution, the supernatant of uncontracted myofibrils to which ATP was added, the supernatant of myofibrils contracted with ATP and the supernatant of ATP-contracted myofibrils to which additional ATP was added. Structurally intact purified myofibrils were free of myokinase and adenylic acid deaminase activity. The supernatant had no ATP-ase activity. During contraction the ATP in the supernatant was converted to ADP by an ATP-ase which could not be separated from the myofibril. There was no passage of ATP-ase into the supernatant during contraction. The ADP resulting from this reaction did not enter into permanent combination with the myofibril during contraction.

Differential dimensional effect of heat and formic acid upon elastic and collagenous connective tissues. J. P. AYER,* SHIELDS WARREN AND GEORGE M. HASS. *Cancer Research Inst., New England Deaconess Hosp., Boston, Mass., and Rush Lab. of Pathology, Presbyterian Hosp., Chicago, Ill.*

Canine aorta immersed in concentrated formic acid extended slightly in all dimensions. Maintained in formic acid at 45° C. the tissue rapidly extended in length and width during the first 36 hours when non-elastic tissues were shown to be extracted. Thereafter the extension became gradual and constant until just before solution it equalled the maximal mechanical extension of whole aorta. The extension of purified elastica was proportional to the concentration of formic

acid. Increasing the temperature of whole aorta resulted in 2 distinct contractions; the first occurring at lower temperatures, the second at 65°. Subsequent re-extensive recoil on cooling was 56%. Purified elastica displayed the primary contraction only with 100% recoil. Increasing the temperature of canine Achilles tendon caused a single explosive contraction of 80% at 65° C. and subsequent recoil of 12%. Prior formalin fixation raised the critical temperature for contraction and increased the recoil to 25%. Fresh tendon in concentrated formic acid contracted 80% with 10% recoil on washing. Prior formalin fixation reduced the maximal contraction but caused 100% recoil. Correlative electron microscopy revealed alteration of the period in the fibrils.

Development of Newcastle disease virus in chick embryo cells. A study with the electron microscope. FREDERIK B. BANG. *Johns Hopkins Univ. School of Medicine, Baltimore, Md.*

Three methods of preparation for electron microscopy have been used and 3 strains of virus studied. The methods are: *1*) infection of the chick embryo chorio-allantoic membrane and subsequent tissue culture, *2*) infection of tissue cultures of membrane and muscle cells and *3*) thin sections of the chorio-allantoic membrane from infected embryos. Epithelial cells, whether in tissue culture or in the embryo, may show normal 'microvilli.' Infected epithelial cells show large numbers of long filaments extending from the surface. These are longer, more profuse, denser than the normal microvilli and often have terminal vesicles. Such filaments, in 4 day old infections in the allantoic sac (Blacksburg strain), resemble a profuse surface growth of fungi. Release of the essentially spherical virus into the allantoic fluid is probably accomplished by a disintegration of these filaments. Epithelial hypoplasis of the cells lining the allantoic sac is a characteristic reaction to this strain of virus, and filament formation precedes destruction of these cells. Fibroblast cells in tissue culture support the growth of all 3 strains of virus. Filament formation in these cells is minimal or absent. Cell destruction leaves scattered spherical virus particles along with much cell debris.

Effects of cortisone on healing of non-perforating wounds of cornea in normal and scorbutic guinea pigs. AELETA BARBER AND W. G. NOTHACKER (introduced by RUSSELL L. HOLMAN). *Dept. of Pathology, Louisiana State Univ. School of Medicine, New Orleans.*

The effects of systemic and topical cortisone on the healing of non-perforating wounds in the cornea of control and scorbutic guinea pigs have been studied. The symptoms of scorbutus in guinea pigs were slightly delayed but not materially altered by daily injections of cortisone in doses of 0.1 mg., 1.0 mg. and 10 mg. The transient infiltration of eosinophils in the cornea which occurs normally during the first 24 hours after operation on the guinea pig was reduced by both cortisone treatment and the vitamin C deficient diet, but was more marked in the animals receiving cortisone. In guinea pigs on control diet, daily injections of 10 mg. cortisone resulted in delayed wound healing occasioned by the belated appearance of fibroblasts and by the prolonged immaturity of collagen. Similar treatment with cortisone in animals on scorbutigenic diet failed to change the effects of vitamin C deficiency; in which case, there was a definite increase in fibroblastic proliferation. One unanticipated finding developed; whenever and wherever contact between regenerating epithelium and collagen occurred, the points of contact were silver positive. The suggestion is offered that this contact between epithelium and collagen produces or yields a reducing substance having an affinity for silver salts.

Measurement of renal gluconeogenesis in dog. JESSE L. BOLLMAN AND JOHN H. GRINDLAY. *Mayo Fndn., Rochester, Minn.*

Samples of renal venous blood were withdrawn at intervals for several days after implanting small plastic tubing in one renal vein. The difference in the renal venous content of sugar, urea and creatinine from that of simultaneously withdrawn arterial blood and the amounts of these substances in the urine furnished the basis of calculations of the blood-kidney-urine exchange of sugar. At normal levels of blood sugar 20 experiments indicated some utilization of sugar by the kidney, 13 indicated more sugar coming from the kidney and 2 showed equal amounts of sugar going in and out of the kidney. At hypoglycemic levels after insulin similar experiments were 5, 3, 1. With continuous injection of glucose to produce hyperglycemia and glycosuria the renal vein blood always contained definitely less sugar than arterial blood. The urinary sugar was less than that abstracted from the blood by the kidney in 10 experiments and only slightly greater in 1. After phloridzin, the urinary sugar was less than that withdrawn from the renal blood in 8 experiments and slightly greater in 2 experiments. These equivocal results may best be explained by factors of time for renal circulation, reabsorption of urinary glucose, and the changing glucose content of arterial blood. They do not support a concept of gluconeogenesis by the dog kidney.

Bowel lesions following single deuteron irradiation. V. P. BOND,* M. N. SWIFT,* C. A. TOBIAS* AND G. BRECHER. *Naval Radiological Defense Lab., San Francisco, Calif., and Natl. Insts. of Health, Bethesda, Md.*

Segments of rat gut were exposed to single

doses of 1800–2800 rep by means of the cyclotron 190 Mev deuteron beam of $\frac{5}{8}$ in. diameter, applied through the intact skin. The beam provides a uniform depth dose and absence of lateral scattering. The biologic effectiveness of 1 rep of the cyclotron beam had been found comparable to 1 r delivered by a 200 kvp x-ray machine in previous lethal-dose studies. The bowel lesions were characterized grossly by destruction of the mucosa and chronic ulceration leading within 5 months to formation of easily palpable discrete annular thickenings in 80% of the surviving rats. Microscopically, lesions from animals killed at 3, 5 and 7 months after irradiation showed, in the ulcerated areas, replacement of the entire muscular wall by granulation tissue and dense fibrous tissue, partly hyaline in appearance, and intimal thickening and proliferation of arteries and veins, with and without hyalinization. These changes resembled those described in chronic radiation injury of the gut, except for the absence of abnormal fibroblasts. From the 5th month on, there was marked proliferation of the mucosal epithelium at the edge of the ulcers, with formation of well differentiated mucus producing glands, frequently extending into the muscularis. Metaplastic bone formation was occasionally observed in proximity to proliferating glands. Invasion of lymphatics or of lymph nodes was not found in the present series and further studies are needed to determine whether the lesions are neoplastic.

Effect of sodium monofluoroacetate on bacterial metabolism of citrate in human blood. ABRAHAM BRAUDE, JAY SANFORD AND JANE BARTLETT (introduced by C. C. STURGIS). *Dept. of Internal Medicine, Univ. of Michigan Med. School, Ann Arbor.*

Citrated human blood was clotted by 125 strains of 14 species of bacteria. Proof was obtained that clotting was due to consumption of citrate by destroying with growing bacteria the anticoagulant action of solutions of citrate before adding blood. Frequency of clotting within a species was parallel with its ability to utilize citrate. Thus 16 of 18 strains of *A. aerogenes* produced clotting in contrast to 12 of 20 strains of *E. coli* and 4 of 13 strains of enterococcus. Clotting of citrated plasma and blood by enterococcus and *A. aerogenes* was inhibited by sodium monofluoroacetate, a substance which did not reduce bacterial growth or possess anticoagulant properties in the concentration used. It has been postulated that sodium monofluoroacetate inhibited oxidation of citrate in the tricarboxylic acid cycle. As enterococci lack cytochromes, another mechanism of inhibition must be sought for this species. Enterococci have been found to produce acetic acid from citric acid with intermediate and successive formation of oxaloacetic and pyruvic acids. These results are compatible therefore with the premise that fluoroacetate competes with acetate ion. In the case of *A. aerogenes* also, utilization of citrate and inhibition of utilization by fluoroacetate need not involve oxidation via the tricarboxylic acid cycle. Thus *A. aerogenes* clotted citrated blood in the complete absence of oxygen as readily as in its presence. Furthermore, inhibition of clotting by fluoroacetate is reflected in strikingly similar curves for *A. aerogenes* and enterococcus.

Comparative study of clotting defects in human, canine and porcine hemophilia. K. M. BRINKHOUS, F. C. MORRISON, JR* AND M. E. MUHRER.* *Dept. of Pathology, Univ. of North Carolina, Chapel Hill, and Dept. of Agricultural Chemistry, Univ. of Missouri, Columbia.*

Previous work indicated that the clotting defects in canine and human hemophilia are identical. In each the prolonged clotting time and impaired prothrombin utilization are corrected by small amounts of normal plasma. In the hemophilia-like disease of swine (HOGAN AND MUHRER, 1941) the prolonged clotting time is corrected by normal swine blood. Prothrombin utilization tests in bleeder swine blood by the 2-stage method showed variable results; in some animals, no prothrombin was utilized in 90 minutes, while in others, prothrombin utilization approached that of normal swine blood. Plasma antihemophilic factor (AHF) assays were done by Graham's method on normals and bleeders of each species. Using normal dog plasma as the standard, human plasma contained roughly $\frac{1}{5}$ as much AHF, and swine plasma $1\frac{1}{2}$–2 times as much. Bleeder dog and bleeder human plasmas contained no detectable AHF, while bleeder swine plasma varied from none to small amounts, with an average of about 6% AHF. Both human and canine hemophilic bloods were used as substrates. The strong corrective action of all the normal plasmas on the clotting defect of the hemophilic bloods, and the lack of such effect between the hemophilic bloods suggest that a similar clotting anomaly exists in all 3 diseases. The anomaly in the porcine disease appears less severe than that in the other 2 species.

Siderosis and mast cell vacuolation in albino rat after toluidine blue O administration. PERIHAN CAMBLE (introduced by JACOB FURTH). *Cancer Research Lab., Univ. of Florida, Gainesville.*

Twelve rats (Sprague-Dawley-Holtzman) were injected 3 times weekly (i.p.) with 1 ml. 0.5% toluidine blue (3 and 9 injections). Erythrocyte count range of controls, 5,620,000–7,380,000; that of the treated group: 5,860,000–6,800,000. Hemoglobin ranges were 109–133 and 103–120 respec-

tively. Only 4 experimental rats showed polychromasia. The fragility test revealed no alteration. Two rats developed peritoneal hemorrhages. One of these showed a hematoma at injection site, the other hemorrhagic erosions of the gastric glandular mucosa, jejunal hemorrhagic infarction, hemorrhage in the urinary bladder. The principal histopathologic lesion was diffuse siderosis of spleen, lymph nodes. Mast cells were studied in dermis, stomach wall, mesentery, heart, lymph nodes, thymus, spleen. They showed depletion of their metachromatic granular substance and metachromasia of their vacuolated cytoplasms. Sometimes mast cells with these characteristics were observed in the widened sinuses of the lymph nodes. Occasionally, few intracellular metachromatic clumps and/or granules, and extracellular granular material were observed. The results partly confirm and extend those of Dunn (*Federation Proc.* 8: 353, 1949) in mice, and those of Stolarsky and Haley in rabbits (ibid. 10: 337, 1951). They indicate that toluidin blue effects erythrocyte destruction, slight hemoglobin reduction, and release of the granular substance (heparin) of mast cells which then exhibit the features of Paff and Bloom's degenerated mast cells (*Anat. Rec.* 104: 45, 1949) at the end of their secretory heparin cycle. Hemorrhagic lesions are effects of the resulting hyperheparinemia.

Desoxyribonucleic acid (DNA) content of tumor cells determined microphotometrically. WILLIAM H. CARNES, NORMAN WEISSMAN AND BENJAMIN GOLDBERG.* *Depts. of Pathology, Physiological Chemistry and Preventive Medicine, Johns Hopkins Univ. Med. School, Baltimore, Md.*

Published data of several investigators indicate an increased DNA content of tumors compared to normal homologous tissues. We have determined the relative DNA content of individual cells of the transplantable mouse tumors, Sarcoma 180 and lymphosarcoma L1, by the microphotometric measurement at 546 mμ of the Feulgen nucleal reaction on cells isolated in sucrose and fixed in formalin as described by Pollister and Ris (*Cold Spring Harbor Symp. Quant. Biol.* XII: 147, 1947) and Ris and Mirsky (*J. Gen. Physiol.* 33: 125, 1949). The results show a high individual variation in DNA content of the nuclei. Between 80 and 90% of the relative values so determined fall within the extremes of a fourfold difference. The values tend to cluster into groups corresponding approximately to the polyploid classes of homologous normal liver cells similarly measured. The results are consistent with the observations of others on the occurrence of polyploidy and polyteny in mammalian tumors and indicate a high frequency of departure from the normal diploid DNA content in these cells. The average DNA content of random samples of tumor cells falls in the range of that of tetraploid hepatic nuclei. Chemical analyses (Weissman *et al.* to be published) of counted cellular suspensions of the tumor cells show an average cellular DNA content of the order of 10^{-8} mg. From this it may be deduced that the bulk of the tumor cells contained between 5×10^{-9} and 2×10^{-8} mg. DNA.

Importance of disulfide (-S-S-) linkage in blood clotting mechanism. JOHN R. CARTER AND E. D. WARNER. *Dept. of Pathology, State Univ. of Iowa, Iowa City.*

By introducing a variety of alkylating agents, oxidizing and reducing agents, sulfhydryl and organo-metallic compounds into the 2-stage clotting system, we have shown that those compounds which alter the integrity of the disulfide linkage caused significant inhibition of clotting as determined by a decrease in thrombin yield. Conversely, compounds which are commonly used to inhibit or block sulfhydryl groups produced little or no inhibition of clotting. Additional experiment showed that the inhibitors of the disulfide linkage, in the molar concentrations used, exerted their effect by acting on the substrate, prothrombin, and not on thrombin, fibrinogen, thromboplastin, or Ac-globulin. When the plasma-inhibitor mixture was dialyzed against buffered saline, partial reactivation or 'de-inhibition' was accomplished as determined by the recovery of thrombic activity. This would indicate that the effect of the disulfide inhibitors is reversible. When an oxidizing agent such as hydrogen peroxide was added in increasing concentrations to a system which was already inhibited by a sulfhydryl compound there was gradual recovery of thrombic activity followed again by a decrease of activity as the concentration of hydrogen peroxide was increased further. Thus, the effect of the inhibitor can be shown to be reversible by oxidation.

Identification of metastatic prostatic carcinoma. GEORGE W. CHANGUS. *Inst. of Pathology, Univ. of Tennessee, Memphis.*

Previous investigators have not been able to consistently demonstrate significant histochemical acid phosphatase activity in carcinoma metastatic from the prostate gland. Fixatives, heat, embedding and deparaffinizing agents have been shown to variably inhibit the acid phosphatase activity. This study indicates that relatively uniform results can be obtained by the use of the routine frozen section method. Frozen sections (about 15 microns thick) of unfixed tissues from 34 metastatic carcinomas were placed on slides, allowed to partially dry for 15–20 minutes at room temperature, and then incubated in the glycerophosphate buffered mixture at pH 5 for the dem-

onstration of acid phosphatase. All 9 metastatic prostatic carcinomas confirmed by autopsy or prostatic resection showed evidence of abundant acid phosphatase activity after 2–15 minutes incubation even in the absence of elevated acid phosphatase activity in the plasma. Twenty-five metastatic carcinomas arising from organs other than the prostate gland (lung, pancreas, liver, stomach, intestine, urinary bladder, kidney and breast) showed no activity in 15 minutes and little or no activity after 30 and 60 minutes incubation. Histochemical localization of acid phosphatase may be used as an adjunct procedure for the identification of carcinoma metastatic from the prostate gland.

Iron absorption in normal, anemic and hemochromatotic subjects. R. B. CHODOS,* J. F. ROSS, M. A. ADAMS* AND M. C. O'BRIEN.* *Radioisotope Unit and Med. Service, Cushing Veterans Administration Hosp., Framingham, Mass.*

Aside from actual blood loss, there is no mechanism in man to provide for more than minimal excretion of iron. It appears, therefore, that iron equilibrium in the body is maintained by the regulation of absorption. The mechanism of this regulation is best explained by the apoferritin-ferritin system developed by Hahn and Granick. According to this concept, iron is taken up by the mucosal cells until all the cellular apoferritin is converted to ferritin. Further iron is not absorbed until some of the ferritin iron has been transferred to the plasma. Data in the literature indicate that this mucosal block system is not a complete regulator. Our investigation was undertaken to study iron absorption in normal, anemic, and hemochromatotic subjects. Radioactive iron-59 was administered orally. The unabsorbed portion of this dose was recovered in the feces and measured by gamma counting techniques. The iron-59 incorporated in hemoglobin was also determined. That portion of the dose not thus accounted for was presumed to have been absorbed and deposited in the body store of iron. Data obtained revealed that absorption ranged from 1–16% in normal and hemochromatotic subjects. Non-iron deficient anemic subjects absorbed approximately the same amount. Iron deficient subjects absorbed 20–60%. These observations suggest that the body iron stores may be a controlling factor in iron absorption.

Platelet studies in hemorrhagic phase of acute radiation injury. E. P. CRONKITE* AND GEORGE BRECHER. *Naval Med. Research Inst. and Natl. Insts. of Health, Bethesda, Md.*

The correlation between bleeding tendency, platelet levels, and *in vitro* coagulation tests was studied in dogs exposed to a uniformly lethal dose of 600 r (2.0 mev x-ray). At death, hemorrhage was usually widespread throughout the body and uniformly present in the lymph nodes. Heparin-like anticoagulants were not found. Fibrinogen, prothrombin, accelerator globulin and antihemophilic factor were present in normal or increased amounts and serum prothrombin conversion accelerator could be evolved when coagulation was forced. The bleeding tendency paralleled the degree of thrombocytopenia. Prothrombin utilization in the course of spontaneous coagulation diminished proportional to the fall in the platelet count. The clotting time of whole blood progressively increased as the platelets fell below 100,000/mm.^{3}. Virtually no prothrombin was utilized during a 60 minute period at platelet levels below 10,000/mm.^{3} and whole blood clotting times became greatly prolonged. All of the coagulation defects were corrected by the addition of as little as 8,000/mm.^{3} separated platelets *in vitro*. In the living animal, by contrast, the platelet levels attained by transfusion of separated platelets had to be brought up to at least 180,000/mm.^{3} in order to correct all coagulation defects. However, spontaneous bleeding was prevented at levels of transfused platelets that did not correct all coagulation defects. Dogs that received less radiation and survived were studied through the recovery stage. During this stage, the whole blood clotting time and prothrombin utilization returned to normal at surprisingly low platelet counts (20–40,000/mm.^{3}). Concomitantly bleeding ceased.

Bi-phasic nature of renal calcification. LEWIS K. DAHL AND VINCENT P. DOLE (introduced by EUGENE L. OPIE). *Hosp. of Rockefeller Inst. for Med. Research, New York City.*

Pathological calcification can be produced in the kidneys of rats by the injection of uranium nitrate. (DICKSON. *Arch. Int. Med.* 3: 375, 1909.) Since the mature calcified lesion is the end stage in a series of chemical reactions, it is evident that the primary chemical changes must be elucidated before the mechanism of abnormal calcification may be understood. Under standard conditions, the lesion produced by uranium in the rat kidney evolves in a relatively predictable fashion, which thus allows studies to be made prior to or during the early phases of calcification. By the combined application of histological and micro-analytical procedures, a series of 16 control and 33 uranium treated rats was studied. It was demonstrated that the process of calcification was divisible into 2 components: a primary accumulation of calcium in association with an unidentified anion, and a secondary conversion of this complex into a precipitate of calcium phosphate. Except for the exclusion of chondroitin sulfate, the nature

of the anion present during the primary accumulation remains unidentified.

Homotransplantation of lung. H. A. DAVIS, J. P. O'CONNOR,* G. J. COLOVIRAS, JR* AND D. A. STRAWN.* *Dept. of Surgery, College of Med. Evangelists, Los Angeles Div., Los Angeles, Calif.*

The fact that tissue homotransplants do not ordinarily survive permanently has stimulated considerable interest in the factors underlying the destruction of such homotransplants. Since homotransplantation of the lung has heretofore not been performed, it has not been possible to investigate the functional and structural behavior of transplanted pulmonary tissue. To study this problem, a series of 34 dogs was used. After considerable difficulty a technic was developed which permitted suture anastomosis of the bronchus, pulmonary artery, superior and inferior pulmonary veins of the transplanted lung and those of the recipient animal. The left lung was transplanted in all experiments, and the operation was successfully completed in 4 animals. Following operation the recipient dogs inflated both lungs synchronously but exhibited periodic attacks of dyspnea lasting 1–2 minutes. The structural changes in the transplanted lungs in the order of their appearance were edema of the alveoli, invasion of the pulmonary tissue by lymphocytes and polymorphonuclear leukocytes and necrosis of the alveolar walls. Cellular infiltration, vascularization of the alveolar septa and air-containing alveoli were most evident near the pleural surfaces where adhesions had formed to the pareital pleura and pericardium. Since technical errors were responsible for the subsequent deaths of the dogs having the homotransplanted lungs, correction of such errors will probably prolong the survival times of these animals. In this way the last functional and structural alterations of the homotransplanted lung can be ascertained.

Histochemical reactions demonstrating protein in Charcot-Leyden crystals. CLYDE J. DAWE* AND W. LANE WILLIAMS. *Sec. on Surgical Pathology, Mayo Clinic, Rochester, and Dept. of Anatomy, Univ. of Minnesota, Minneapolis.*

Charcot-Leyden crystals were studied in smear preparations from *a*) formalin-fixed stool and *b*) buffy coats treated with detergent and airdried to slides. Stool crystals gave positive xanthoproteic, Millon, diazo, arginine and tryptophane reactions. Basophilia was unaffected by ribonuclease and saliva. Crystals stained deeply with gallocyanin-chromalum at pH 2.5 and 3.9 gave a positive Schiff-reaction which was decreased by Feulgen hydrolysis, and were only weakly positive after 14 hours treatment with normal HCl or 0.3N tricloroacetic acid for 15 minutes, both at 90°C. Such treatment did not affect affinity for toluidine blue or acid or basic fuchsin, but stronger acid treatment diminished the uptake of these dyes. Air-dried buffy coat crystals gave negative, Schiff, Feulgen, and P-A-S reactions, while other reactions were comparable to those of formalin fixed stool crystals. Air-dried buffy coat crystals were not Schiff positive after exposure to desoxypentosenucleic acid hydrolysates in acid or neutral solution, or to formalin. Formalin-fixed buffy coat crystals appeared unaffected by pepsin, trypsin, and chymotrypsin. The apparent morphologic origin of Charcot-Leyden crystals from eosinophil leukocyte nuclei and their reputed limitation to primates suggest protein content of nuclear derivation. Their amphophilic (staining) properties are compatible with ability to bind either nucleic acids or protamines. The presence of arginine, tyrosine, and tryptophane, and affinity for developed leuko-fuchsin and iron hematoxylin are features in common with the nuclear protein chromosomin described by the Stedmans.

Influence of hepatic injury upon choline utilization. J. DE LA HUERGA,* HANS POPPER AND DIETER KOCH-WESER.* *Hektoen Inst. for Med. Research and Dept. of Pathology, Cook County Hosp. and Northwestern Univ., Chicago, Ill.*

Since the urinary trimethylamine excretion following choline intake is reduced in human hepatic diseases, the type of injury causing the depression was studied in rats with various hepatic injuries. Previously it was shown that of a test dose of choline given, 60% in the human and 40% in the rat are transformed by some intestinal bacteria into trimethylamine and excreted in the urine. Only about 20% of fed choline is excreted as trimethylamine by rats with chronic hepatic injury produced by butter yellow, ethionine, bromo-benzene, carbon tetrachloride and by a high fat-low protein diet. The excretion was normal in acute hepatic injury and during experimental biliary obstruction. The depression was not related to hepatic fatty metamorphosis since it persisted if fat was removed. It was not related to choline deficiency. Drugs influencing intestinal motility were without effect. The stool did not contain demonstrable choline even with depression of the trimethylamine excretion. It appears, therefore, that chronic hepatic injury as such affects the degree of choline transformation in the intestinal tract either by causing increased choline absorption in the upper intestine before the bacteria are able to transform choline or by primary depression of choline transformation by intestinal bacteria. The latter effect would be similar to the depression of trimethylamine excretion resulting

from oral administration of even small doses of antibiotics. The influence of hepatic injury upon intestinal processes is under further study.

Interrelationship of experimental coronary lesions and altered lipid metabolism. G. I. DESUTO-NAGY AND L. L. WATERS (introduced by HARRY S. N. GREENE). *Dept. of Pathology, Yale Univ. School of Medicine, New Haven, Conn.*

Acute, focal injuries of the coronary arteries, produced by injections of allylamine, were shown to become selective sites for accumulation of lipid materials from the bloodstream when the lipid metabolism of the dog was simultaneously disturbed by injections of detergents (DESUTO-NAGY AND WATERS: *Federation Proc.* 10: 353, 1951). The primary, lipid-free coronary injury was thus transformed within a few days or weeks into a lipid-filled, foam-cellular lesion that had many features of early arterio-sclerosis of man. In contradistinction, when high fat diets (butter, lard, Crisco, olive oil) were fed to dogs with acute arterial lesions so that a marked alimentary hyperlipemia resulted, an extracellular accumulation of lipids occurred in the injured segments of the arterial wall with only minimal evidence of phagocytosis. The blood lipid patterns of these 2 types of hyperlipemia were quantitatively and qualitatively different. The detergent-induced hyperlipemia with an increased cholesterol:phospho-lipid ratio in the dog was more sustained than the alimentary one. Histochemical tests for cholesterol were positive only on the foam-cellular lesions. The extracellular fat disappeared from the arterial wall of dogs on high fat diets within a few days or weeks. The lipids in the foam-cellular lesions persisted longer, although even these tended to disappear, in spite of a continuously maintained state of hyperlipemia, within a few months, as the primary coronary injury healed with fibrosis.

Radioactive zinc metabolism in patients with neoplastic diseases. FRANKLIN G. EBAUGH, JR.* AND JOSEPH F. ROSS. *Evans Memorial, Massachusetts Memorial Hosps. and Dept. of Medicine, Boston Univ. School of Medicine, Boston.*

Zinc is known to be an essential element in all forms of living matter and to be an integral component of certain enzyme systems. Others have shown that there are differences in zinc content between normal and neoplastic tissues. The purpose of this study was to explore zinc metabolism in patients with malignant diseases. Patients were given radioactive zinc ammonium citrate by intravenous injection. The turnover rates of radioactive zinc were determined for the erythrocytes, leukocytes, and plasma. Simultaneous observations were made on the rate and route of excretion of the radioactive zinc. When possible the distribution of radioactive zinc in tissue obtained post mortem was ascertained. The liver contained several times as much zinc as did any other tissue. Zinc is rapidly cleared from the plasma; only 11% of the administered dose remained after 30 minutes. The leukocytes took up twice as much zinc/cc. as did the erythrocytes. Analysis of the leukocyte uptake curves yielded information on the maturity time and life span of the leukocytes. Thus far there have been no significant differences between the initial rate of uptake of zinc by leukemic and normal leukocytes.

Influence of cortisone and of newborn state on host resistance to transplanted heterologous tumors. E. J. EICHWALD, H. Y. CHANG AND M. LETTIERI (introduced by F. D. GUNN). *Dept. of Pathology, Univ. of Utah College of Medicine, Salt Lake City.*

Tumors can be successfully transplanted heterologously to certain sites of low natural resistance (anterior chamber, brain, subdural space, etc). Mouse Sarcoma 37 will grow in the anterior chamber of the guinea pig for about 2 weeks; after this period it regresses invariably, indicating the development of a resistant state. It follows that the tumor faces both a natural and an acquired resistance. Factors influencing either type of resistance will modify growth of the tumor. An increase in growth of transplanted tumors has been reported following cortisone administration, and also when newborn instead of adult hosts were used. To determine whether this increase is due to a lowering of the natural or the acquired resistance, Mouse Sarcoma 37 was transplanted to the anterior chamber of newborn guinea pigs, adult controls, and adult guinea pigs under cortisone medication. The animals were sacrificed at regular intervals and the amount of tumor tissue present measured by histological means. Up to the 10th day the amount of tumor tissue in the newborn animals significantly exceeded that in the controls and cortisone treated animals. Regression occurred at about the same time as in the controls. In the cortisone treated animals the amount of tumor at the end of the 2nd week significantly exceeded that in the controls in which regression had already begun. It is concluded that the natural resistance to transplanted heterologous tumors is lower in the newborn state, while the acquired resistance is impeded under cortisone administration.

Pathological changes in vagina and cervix of mice with advancing age and under influence of injections of alpha estradiol-sensitized collodion particles. CYRUS C. ERICKSON, WILLIAM L. WILLIAMS, JR.* AND KATHERYN PAULLUS.* *Inst. of Pathology, Univ. of Tennessee, Memphis.*

Suntzeff *et al.* (*Am. J. Cancer* 32: 256, 1938) and Gardner and Allen (*Yale J. Biol. and Med.* 12: 213, 1939) have previously described observations on the influence of administered estrogens and advancing age in several mouse strains; they recorded varying degrees of proliferative vaginal and cervical epithelial changes to carcinoma or carcinoma-like lesions. This preliminary report records findings in a study of the pathological changes occurring in the vagina, cervix and uterus of 320 Bagg Albino mice administered alpha estradiol-sensitized collodion particles (DULANEY, ALDEN AND CONGER, chemically assayed by CONGER). Spayed and non-spayed mice received either approximately 1.1 gamma biweekly or approximately 4.4 gamma monthly; animals were started on injections prior to 10 days. Controls included 86 untreated mice. Animals were killed at monthly intervals to 15 months. Estrogen was administered as a collodion particle preparation in the belief that such injections might provide an *'in vivo* depot' from which the estrogen would be available for slow and continuous absorption and thus test a more constant source of experimentally induced estrogenic stimulation. Vaginal cytologic examinations were done. Epithelial metaplasia, cytologic atypicalities, and carcinomas have been observed microscopically in the vagina and cervix of treated mice. The incidence, degree and extent of atypical epithelial changes will be discussed further and illustrated. Epithelial atypism tends to occur with increased frequency with the increasing duration of treatment and corresponding increase of age.

Histochemical localization of malic, isocitric and succinic dehydrogenases in kidney. EMMANUEL FARBER,* WILLIAM H. STERNBERG* AND CHARLES E. DUNLAP. *Dept. of Pathology, Tulane Univ., New Orleans, La.*

Frozen sections of tissues were stained for several enzymes of the Krebs cycle by incubation with tetrazolium salts ('blue tetrazolium' and neotetrazolium) and appropriate substrates and cofactors. We have confirmed the staining for succinic dehydrogenase, as reported by Seligman and Rutenberg, and have also stained for malic and isocitric dehydrogenases. For malic dehydrogenase, DPN is essential, and either glutamate or cyanide must be added to remove oxalacetate. Fumarase may be indirectly determined by substituting fumarate for malate as substrate and staining for malic dehydrogenase. Isocitric dehydrogenase together with aconitase was demonstrated by using citrate as substrate. For this reaction TPN and Mn^{++} are essential. Cysteine is also necessary for the activation of aconitase. No staining occurred when the tetrazolium salts were reduced nonenzymatically. The specificity of the histochemical stain for succinic dehydrogenase is indicated by the absence of staining when alphaketoglutarate, fumarate or malate are substituted for succinate as substrate. Frozen sections, as used in the present study, like homogenates, appear to require the addition of coenzymes and cofactors necessary for enzyme action. Thus, the demonstration of isolated enzyme activity is possible. Striking patterns of localization were found, particularly in the kidney. With all the enzymes studied, the same 4 zones of localization were regularly seen in the tubules of the kidney of man, rat, cat, hamster and rabbit: high activity in the cortex and midmedulla; moderate activity in the cortico-medullary region and minimal or no activity in the papilla. The glomeruli show no activity.

Significance of plasma iron turnover rate. STUART C. FINCH* AND JOSEPH F. ROSS. *Evans Memorial, Massachusetts Memorial Hosp., and Dept. of Medicine, Boston Univ., Boston.*

The purpose of this study was to determine the physiological significance of the plasma iron clearance rate and its relationship to hemoglobin synthesis, tissue iron, plasma iron levels, and reticulo-endothelial function. This information is of importance in understanding the fundamental physiology of internal iron metabolism, the plasma iron tolerance curve, the erythrocyte radio iron utilization curve, and rates of erythropoiesis. Dogs and human subjects were given tracer amounts of radio iron intravenously, and the disappearance rate of this iron from the plasma was determined by radioactive assay. In animals erythropoiesis was accelerated and depressed, iron reserves were increased and depleted, plasma iron was elevated and depressed, and attempts were made to block reticulo-endothelial function. Corresponding studies in man were made in individuals with alterations in rates of erythrocyte formation and variations in tissue iron reserves. Plasma iron clearance rates determined under these experimental conditions indicate that the rate of hemoglobin synthesis is the primary regulator of plasma iron turnover. However, iron reserves, plasma iron levels, and reticulo-endothelial function exert secondary effects. The relationship of plasma iron turnover rates to the interpretation of plasma iron tolerance curves and rates of erythropoiesis will be discussed.

Effect of specific antibody on metabolism and growth of bacteria. RICHARD H. FOLLIS, JR., JACK M. BURNETT* AND ZELDA E. LASCHEVER.* *Dept. of Pathology, Johns Hopkins Univ. Med. School, Baltimore, Md.*

When *S. marcescens* is grown in trypticase soy broth in the presence of specific antibody, an

appreciable increase in O_2 consumption over control organisms grown in media containing normal serum is observed (*Proc. Soc. Exper. Biol. & Med.* 75: 206, 1950). Antisera to a strain of *S. typhosa* and *Sh. paradysenteriae* were produced in rabbits. When these organisms were grown in the presence of their specific, ethanol precipitated antibody, an increase in O_2 consumption similar to that already observed with *S. marcescens* was demonstrated. This phenomenon may only be observed with growing organisms during the logarithmic phase; washed or older bacteria fail to show any difference from controls. The increased metabolism measured manometrically appears to be due to the appearance of an increased number of organisms. When the contents of the Warburg flasks are centrifugalized at high speed, washed, and recentrifugalized, the sedimented bacteria grown in the presence of specific antibody have a higher N and P content; in addition, suitable hydrolysates of organisms grown in antibody exhibit an increase in ultraviolet absorbtion, measured at 2600A, over the controls. Complement has no effect on the difference observed. Organisms which are grown for a number of generations in specific antibody are not mutated in any way since the O_2 consumption in the absence of antibody is similar to that of controls. When a synthetic medium containing crystalline amino acids in the proportion of those found in casein is substituted for trypticase soy broth, O_2 consumption is again increased in the presence of antibody. Work is in progress on inhibition of the difference by removal of certain amino acids from this mixture. (Supported in part by grants from the John and Mary Markle Foundation and the Division of Research Grants and Fellowships, U. S. Public Health Service.)

Non-enzymatic reduction of basophilia and metachromasia in tissue sections by proteins. JOHN E. FRENCH* AND EARL P BENDITT. *Dept. of Pathology, Univ. of Chicago, and La Rabida Jackson Park Sanitarium, Chicago, Ill.*

Tissue sections fixed in acetone or alcohol were incubated with dilute solutions of protamine, histone, or albumin at pH's below the respective isoelectric points of these substances. Subsequent staining with thionin or toluidine blue showed that tissue basophilia and/or metachromasia were reduced or abolished by this procedure. This effect was dependent upon the length of incubation and the concentration of protein and appeared to have a pH optimum. The firmness of binding of the substances varies with pH, ionic strength, residual charges of the protein and other factors. The change in basophilia appears to be due to an affinity of these proteins for acidic tissue components which is greater than that of the basic dyes. These facts indicate that in procedures involving the application of crude enzyme solutions to tissue sections, reduction in basophilia or metachromasia may be due in part to non-enzymatic factors. Furthermore, basic protein groups already present in the tissue may influence the degree of basophilia quite apart from any quantitative change in so-called basophilic components.

Fulminant experimental blastomycosis produced by employment of Roentgen radiation and cortisone. JACK FRIEDMAN,* FRANK J. ROTH, JR.,* ALVAR A. WERDER* AND JEROME T. SYVERTON. *Dept. of Radiology, Mt. Sinai Hosp., and Dept. of Bacteriology and Immunology, Univ. of Minnesota, Minneapolis.*

As part of an extensive investigation of the effects of radiation and cortisone acetate (Cortone, Merck) upon infectious agents, these 2 agents singly and in combination were found to enhance remarkably the pathogenicity of *Blastomyces dermatitidis*. One hundred sixty adult Swiss albino mice, CFW strain, were employed in 10 groups of 16 each as test and control animals. The radiation factors were: 60 cm. target-skin distance; 250 kv.; 15 ma.; filter 0.5 mm. copper, 1 mm. aluminum; HVL 1.35 mm.^{3}; size of field, 14.0 diameter. The output as determined by a Victoreen r-meter was 41.5/min. in air. Each test mouse was prepared by whole body irradiation, 200 r or 400 r and/or cortisone 24 hours later in a dose of 2, 4, 6, or 7.5 mg. by injection subcutaneously, and finally, by the injection intraperitoneally of 1.0 ml. of a 1:150 suspension of a 5-day old, yeast-phase culture of *Blastomyces dermatitidis*. An optimal effect was obtained when x-ray, 400 r, and cortisone, 4 mg., were employed. For example, the 16 animals that received the 2 agents in combination died in from 5–12 days to result in an average survival time of 7.3 days for this group. The results for the other 3 groups that received the test infective dose of *Blastomyces dermatitidis* were *1*) fungus only, survival of 11 out of 16 at 40 days and death of 5 in from 13–40 days; *2*) x-ray, 400 r, death in from 4–37 days, average 15.4 days; *3*) cortisone, 4.0 mg., death in from 5–29 days, average 11.3. Since this preparative treatment operates similarly to render mice susceptible to other pathogenic fungi, it is apparent that mice made ready by a single dose of x-ray and cortisone may be utilized advantageously as the host animal for diagnostic and experimental studies.

Effects of steroids on thymus. N. B. FRIEDMAN, E. J. BOMZE* AND S. ROTHMAN.* *Div. of Labs., Cedars of Lebanon Hosp., and Dept. of Pathology, Univ. of Southern California School of Medicine, Los Angeles.*

Pellets of testosterone propionate, estradiol dipropionate, desoxycorticosterone acetate, cortisone acetate and cholesterol were implanted directly into the thymus of rats. Testosterone and cortisone, like adrenal transplants, caused only local involution and evoked a cellular reaction indicating turnover of lipoid materials, Estradiol, on the other hand, resulted in diffuse thymic atrophy without local involution. Cholesterol and desoxycorticosterone were without effect. The results suggested that the estrogen, unlike the androgen and the corticoid, exerted its effect on the lymphoid tissue without itself being altered. There may be more than one mode of action in the involution of the thymus by steroids. Effects on the thymus are not necessarily paralleled in the lymph nodes. (This investigation was supported by a research grant from the National Institutes of Health, Public Health Service.)

Effect of aureomycin and whole blood separately and in combination on x-irradiated dog. F. W. Furth,* M. P. Coulter,* R. W. Miller,* J. W. Howland and S. N. Swisher.* *Univ. of Rochester Atomic Energy Project, Rochester, N. Y.*

In order to evaluate the therapeutic effects of aureomycin and whole blood transfusion, separately and in combination on the acute radiation syndrome, a total of 96 dogs was exposed to 575 r of whole body x-irradiation. The dogs were separated into 4 equal and comparable groups. One group served as controls and received no therapy. Of the 3 remaining groups, 1 received auroemycin orally (approximately 50 mg/kg/24 hr.) in 2 divided doses at 12 hour intervals starting 24 hours after exposure to the irradiation. One group was given whole blood transfusions in 125 cc. amounts whenever the individual dog's hematocrit fell below 80% of its preradiation value. The 4th group received both forms of therapy. No statistically significant difference in rate of mortality or 28 day mortality among the 4 groups was observed. A total of 140 transfusions was administered to 40 dogs for an average of 3.5 transfusions/dog (approximately 50% of the estimated total blood volume). The peak demand for transfusion was on the 14th and 15th days postradiation. In the blood treated groups the average hematocrit was maintained at 80% of preradiation value by means of the transfusions. The pathological data suggest that a higher incidence of pulmonary hemorrhage and edema occurred in the blood treated dogs, indicating a potential hazard in the administration of whole blood during certain of the acute radiation syndrome. (Based on work performed under contract with the U. S. Atomic Energy Commission.)

Distribution of thromboplastic qualities in cell components or normal and neoplastic tissues. LiseLotta Graf, Maurice M. Rapport and Frank Maltaner (introduced by Gilbert Dalldorf). *Div. Labs. and Research, N. Y. State Dept. Health, Sloan-Kettering Inst. for Cancer Research, New York City.*

Cells of liver, spleen, kidney, and lung of normal rats as well as tumor cells from rat and human sources were investigated. Cell components were separated by fractional centrifugation. Isotonic sucrose solution was used as the suspending medium and washing fluid. Thromboplastic activity of the mitochondrial, microsome, and supernate fractions was compared quantitatively with that of the thromboplastic lipide (a preparation of phosphatidyl serine) by a technic described elsewhere (M. M. Rapport and F. Maltaner, this issue). Marked differences were observed in activities of cell components from normal and neoplastic tissues. (Supported in part by a cancer control grant from the National Cancer Institute, U. S. Public Health Service.)

Significance of heterologous transplantability of human cancer. Harry S. N. Greene. *Yale Univ. School of Medicine, New Haven, Conn.*

A series of 123 human tumors were studied with respect to heterotransplantability. Sixty-five of the tumors grew on heterologous transfer while 58 failed to survive. The groups so formed showed no consistent variation in morphology but differed with respect to their behavior in the patient. The heterotransplantable group was associated with a rapidly fatal course while patients with non-heterotransplantable tumors lived for considerable periods of time. There is evidence to suggest that heterotransplantability differentiates different phases of tumor development rather than different categories of neoplastic disease and that the non-heterotransplantable tumor represents an early developmental phase and the transplantable tumor a later stage.

Some chemical properties of nephrotoxic antigen. Seymour A. Greenspon,* Frederick W. Bollinger* and Cecil A. Krakower. *Dept. of Pathology, Univ. of Illinois, Chicago.*

Sixty % trichloracetic acid (TCA) will dissolve isolated canine renal glomeruli at 25° C. after a number of hours. If its action is restricted to 5-6 hours at 25° C. and overnight at 0° C., enlarged glomeruli with petal-shaped tufts are obtained which are microscopically free of cells and nuclei and in different trials are absolutely or almost free of phosphorus and desoxypentosenucleic acid chemically. Such bare glomeruli titer for antigen at the same level as glomerular basement membrane (GBM) obtained by physical means (*Arch. Path.* 51: 629, 1951). This establishes the locus of the antigen within the GBM. Chemically, GBM obtained by sonic vibration is collagenous, being soluble in boiling acetic acid, readily digestible

by pepsin, and slowly and incompletely by trypsin and papain. TCA-treated glomeruli, freed of cells, are instaneously dissolved by pepsin, trypsin, and papain. GBM and its antigen are soluble to a limited degree in 0.04–0.2% acetic acid, even after neutralization and centrifugation at 40,000 rpm. for 2 hours or more; in borate buffer at pH 8.0–9.0; in ½ saturated calcium hydroxide; and by brief digestion with papain. Phosphate buffers at pH 5.0, 7.4, and 9.0 appear to destroy the antigenicity of GBM. Protein denaturants and proteolytic enzymes such as 0.5–1.0 N sodium hydroxide, 50% urea, pepsin, and trypsin destroy the antigen. It seems likely that the antigen is an integral part of the collagen which forms the GBM and specifically, its protein moiety.

Relationship of structure and oxidative phosphorylation in mitochondria of heart muscle. JOHN W. HARMAN AND MURIEL FEIGELSON.* *Dept. of Pathology, Univ. of Wisconsin Med. School, Madison.*

Efficiency of myocardial oxidative enzyme systems as an integrated complex depends not only on the adequate presence of component enzymes but also on the structural integrity of the cytological units which contain the system. The osmotic environment is a paramount factor for the maintenance of the mitochondria and their oxidative capacity in the oxidation of citric acid cycle intermediates. Studies of relationships between oxidation and internal structure of mitochondria permit formulation of conditions which precisely define the interdependence of form and respiration. It is established that oxidation contributes to the maintenance of mitochondrial form by the mechanism of oxidative phosphorylation. The critical influence of oxidative phosphorylation on form is emphasized by the rapid degeneration which occurs when: *1*) phosphorylation is uncoupled by dinitrophenol, Janus Green and usnic acid, which lower P/O ratios significantly: *2*) the high energy phosphate bonds are filtered off through kexokinase into glucose monophosphate; and *3*) ATPase action is not inhibited. On the contrary, conditions which protect the transfer of ~P protect mitochondrial form and phosphorylation. It is significant that with both kexokinase and creatine transphosphorylase, oxidation is augmented several fold. This indicates a mutual influence of oxidation and phosphorylation, manifested by the intervention of secondary phosphate acceptors. Unrestricted play by secondary acceptors is, however, not physiological, as rapid degeneration of mitochondria suggests, when they operate in the system. This type of degeneration is a cytological counterpart of cloudy swelling.

Diagnostic significance of fatty cysts in livers of human autopsy material. W. STANLEY HARTROFT. *Dept. of Pathology and Banting and Best Dept. of Med. Research, Univ. of Toronto, Canada.*

In livers of choline-deficient rats, fat is deposited initially within individual parenchymal cells. As lipid accumulates further, the cells become transformed into thin-walled sacs of cytoplasm which enclose large fat-spherules. Limiting membranes of adjacent cells, so altered, are compressed to tenous septa by contiguous lipid spherules. Still further accumulation of fat may overtax the storage capacity of cells so that these septa are stretched beyond the breaking point. This permits escape and coalescence of the lipid spherules which collectively are now enclosed by the conjoined remnants of cytoplasm of the ruptured cells. Thus the released fat is encysted by the altered cells from which it escaped. Fatty cysts, so formed, are therefore pathognomonic of excessive and damaging degrees of localized fat-storage. The pathogenic role of fatty cysts in dietary cirrhosis has been reported previously (*Am. J. Path.* 27: 951, 1951). Cysts may remain for long periods after the factors responsible for their formation have ceased to operate. At autopsy, fatty cysts have been encountered most frequently in livers (often almost free of other abnormal lipid) of alcoholic and diabetic patients. These residual cysts may be important diagnostic clues in cases of cirrhosis of unknown etiology. From studies of autopsy material, the diagnostic significance of fatty cysts will be assessed. (This work was aided by a grant from the National Cancer Institute of Canada.)

Mechanism of localization of vital dyes in inflamed tissues. HOWARD C. HOPPS AND E. PHILIP KLEIN.* *Dept. of Pathology, Univ. of Oklahoma School of Medicine, Oklahoma City.*

In continued studies of the mechanism of localization in disease the vital dye trypan blue was separated into 4 components by paper chromatography. By column chromatography 2 of these fractions were secured in sufficient amounts for *in vivo* experiments. Their patterns of localization were compared with the whole complex of trypan blue in different inflammatory lesions produced in rabbits. Tissue sections of these lesions were made by the freezing-drying technique and studied with bright-field and phase microscopy to demonstrate the form and location of the localized dye. Reticulo-endothelial organs were also studied.

Localization of Na_{22} in rat kidney. ROBERT B. JENNINGS AND JACOB S. KRAKUSIN (introduced by WILLIAM B. WARTMAN). *Depts. of Pathology and Biochemistry, Northwestern Univ. Med. School, Chicago, Ill.*

Male and female Sprague Dawley rats of varying weight were injected intravenously or intraperitoneally with 1–2 cc. Na_{22} in physiologic saline solution. At varying time intervals (3–70 min.) the renal pedicle was clamped and a thin (2 mm.

thick) hemisection frozen immediately in isopentane at liquid nitrogen temperatures (−160° to −190°C.). After freezing, the tissue was dehydrated at −40°C. under a vacuum of 10 microns for 48–72 hours. The dry specimen was then brought to room temperature in a dessicator, vacuum embedded in paraffin, and thin sections cut using the paraffin backing technique. The excess paraffin was removed from the albuminized slide by heat and photoroentgen film placed in close apposition to the section. Radioautographs obtained in this fashion from rats on a normal diet with water *ad libitum* showed the heaviest zone of concentration to be in the medulla of the kidney (chiefly collecting ducts) with a secondary ring of concentration in the peripheral medulla in the region occupied chiefly by the ascending loops of Henle. These medullary concentration zones were accentuated in dehydrated rats or in rats on a sodium deficient diet. No zones of concentration were observed in rats given organic mercurial diuretics. The medullary concentration zones characteristic of the sodium kidney were absent in radioautographs made using P_{32}, I_{131}, Ca_{45}, and diiodofluorescein (I_{131}). These studies suggest that active facultative reabsorption or excretion of Na may be taking place in the medullary zone.

Atherosclerosis using histochemical and fluorescent microscopic methods. WALTER JOEL* AND HOWARD C. HOPPS. *Dept. of Pathology, Univ. of Oklahoma School of Medicine, Olkahoma City.*

A combination stain using histochemical methods for demonstrating neutral polysaccharides (McManus-Hotchkiss) and acid polysaccharides (Hale-Rinehart) has been devised and applied to atherosclerotic aortas. By this method elastic fibers too are visualized, since they stand out as unstained elements against the interfibrillar polysaccharide. In addition, elastic fibers have been studied by conventional methods and by fluorescent microscopy. A very promising method in fluorescent microscopy utilizes acridine orange at pH 5.7. This fluorochrome has an affinity for polysaccharide, especially that in ground substance, and this selectivity corresponds closely with that shown by histochemical methods. Acid and neutral polysaccharide both stain with acridine. Acriflavine may be combined with berberine or primulin, the latter specific for elastic tissue. Studies of lipid in atherosclerotic aortas have been made with the Schulz reaction for cholesterol and sudan stains. Fluorescent techniques have been particularly valuable. These included the phosphine method recommended by Popper and a new technique using benzpyrine (Neils O. Berg) which demonstrates masked lipids, i.e. those conjugated with protein. Partially bound lipids, coarsely dispersed or in form of cholesterol crystals, will also stain by this method. These studies support the view that, along with changes in lipids, alterations in ground substance are also very important in the pathogenesis of atherosclerosis.

Production of cardiac lesions in guinea pig by various mucopolysaccharides. RUSSELL S. JONES (introduced by GEORGE W. CHANGUS). *Inst. of Pathology, Univ. of Tennessee, Memphis.*

This group of experiments is one phase of a study in the mechanism and specificity of experimental cardiac changes in the guinea pig. The valvular changes are of particular significance in that they resemble those occurring in rheumatic fever and may, therefore, aid understanding this disease. Since rheumatic fever is thought to be a disease of collagen and ground substance mucopolysaccharies, the earlier studies on altered collagen have been amplified by the subcutaneous introduction of crude and refined mucopolysaccharides. The cruder products yield the more marked and consistent changes, suggesting that the injected mucopolysaccharides do not produce disturbances in collagen-ground substance. The aortic and mitral valves are predominately involved. With the dissecting microscope, an edematous thickening is observed about the aortic corpora arantii. The thickening advances along the line-of-contact without significant changes at commissures or base. In the mitral valve the border is thickened and the auricular surface is sometimes granular, especially along the line-of-contact. Microsections show the initial valvular change to be a basophilic edema staining as acid mucopolysaccharides. This is followed by proliferation of endothelial cells especially along the line of contact, and by fibroblastic proliferation and collagen increase. Routine microsections of other structures, except the nervous system, were made. Experimental studies thus far indicate that these valvular changes are a marked local manifestation or a milder generalized connective tissue response to diverse agents including some spontaneous diseases in the guinea pig.

Direct local action of thyroxin and thyroxin analogues in anuran tail fin. JANE COUFFER KALTENBACH (introduced by D. MURRAY ANGEVINE). *Dept. of Pathology, Univ. of Wisconsin, Madison.*

Pellets of thyroxin crystals in cholesterol were implanted into the dorsal tail fins of young *Rana pipiens* tadpoles. Local resorption of the fin was induced prematurely to general metamorphosis by pellets containing 40% thyroxin by weight. Pellets containing 20% thyroxin did not induce fin resorption, but those implanted into other

structures, such as the orbit or the larval mouth furnishings, stimulated local metamorphic responses in those structures. This suggests that the tail fin has a higher threshold of response to thyroxin than do many other larval tissues. A method for testing thyroxin-like activity of various compounds directly on peripheral tissues would aid in determining which portions of the thyroxin molecule exert physiological activity. For that reason, pellets containing thyroxin analogues in cholesterol were implanted into the dorsal tail fin. Acrylic acid analogues (kindly supplied by Dr. S. B. Barker) were used, since such compounds stimulate metabolism in mammals (BARKER *et al.*, 1951) and accelerate general metamorphosis in amphibians (FRIEDEN AND WINZLER, 1951). Pellets containing 3,5,3′,5′-tetraiodo; 3,5-diiodo; and 4′-methoxy-3,5,3′,5′-tetrabromo analogues caused local resorption of the fin. The first 2 analogues were effective at lower concentrations than was thyroxin. The experiments demonstrate local resorption of the anuran tail fin by direct action of thyroxin as well as certain acrylic acid analogues of thyroxin and thereby afford a method for testing thyroxin-like activity of analogues directly on peripheral tissues.

Biological effects of Beta-propiolactone. A. R. KELLY* AND F. W. HARTMAN. *Dept. of Labs., Henry Ford Hosp., Detroit, Mich.*

The virucidal and toxicological properties of B-propiolactone (Betaprone) were reported by this laboratory in 1951 (*Federation Proc.* 10: 220, 358, 361, 1951) when the compound was proposed as a candidate agent for sterilizing blood and plasma. Multiple massive transfusions of Betaprone treated plasma and blood (and of its purified degradation products) indicate an excellent toxicity index for the compound in three animal species and man. Eighty-six treated plasma transfusions have been administered to 46 patients, 2 individuals receiving 8 uneventful transfusions each in 2 months. Toxicological characterization of the compound continues. *Antigenicity*: Guinea pigs failed to develop anaphylaxis following attempts to produce both active sensitization to their own treated blood and passive sensitization to treated homologous plasma. Intradermal skin testing of subjects at intervals after transfusion was likewise negative. *Effects of standardized experimental liver damage on the acute toxicity of Betaprone in mice*: The hepatotoxin CCl_4 significantly decreased both the survival time and LD_{50} following i.v. Betaprone. In contrast to its degradation products in treated blood and plasma, at lethal dosages Betaprone itself is hepatotoxic and nephrotoxic (rabbit and guinea pig, not mouse). *Actions of Betaprone degradation products*: Near-lethal dosages of hydracrylic and β-chloropropionic acids produce peripheral vasodilation in the mouse and rabbit. Acrylic acid itself also elicits this effect. Both degradation products, in large repeated doses, depress the platelet count in the peripheral blood of animals.

Effects of virucide Beta-propiolactone and its degradation products on blood platelets. A. R. KELLY (introduced by F. W. HARTMAN). *Dept. of Labs., Henry Ford Hosp., Detroit, Mich.*

A slight but significant depression of platelet count is the only detectable *in vivo* effect on the formed elements of the blood following massive transfusions in animals of β-propiolactone (Betaprone) treated blood or plasma. To explain this fact, since even lethal doses of Betaprone do not alter platelet counts in rabbits, an investigation of the platelet effects of Betaprone degradation products was necessary. Eight 475 mg/kg. injections of β-chloropropionic acid were administered to rabbits in 14 days. Three doses were well tolerated, but the additional injections eventually produced a 90% reduction in platelet count. Maximal reduction following a single injection occurred at approximately 9 hours. Even with marked platelet depression, bleeding and clotting times were only slightly and transiently altered. Each dose administered approximated the amount of β-chloropropionic acid present in 20 l. of Betaprone treated plasma. Large repeated doses of another degradation product, hydracrylic acid, are thrombocytopenic, while single near-lethal injections are not. Using a siliconed *in vitro* system, platelet curves for hydracrylic and β-chloropropionic acids paralleled *in vivo* curves for 12 and 7 hours respectively. Both degradation products are peripheral vasodilators, but absence of platelet effects, with such vasodilators as sodium nitrite, suggests that this apparent platelet depression is not necessarily due to circulatory effects. Bone marrow macrophages appear unaffected by these compounds.

Hepatic injury due to lack of available amino acids. DIETER KOCH-WESER,* HANS POPPER AND J. DE LA HEURGA. *Hektoen Inst. for Med. Research and Dept. of Pathology, Cook County Hosp. and Northwestern Univ., Chicago, Ill.*

The presence of liver cell damage demonstrable by histological and functional methods (decrease of hepatic and serum esterase, increase of hepatic and serum alkaline phosphatase, bromosulphthalein retention) in rats on high fat-low protein diets and its correction by amino acids suggests an effect of nutritional amino acids deficiency. Similarly, administration of the methionine antagonist, ethionine, produced in acute experiments fatty metamorphosis in female rats and in

chronic experiments diffuse liver cell damage with intralobular fibrosis, abnormal regeneration of liver cells and cholangiolar and reticuloendothelial proliferation (associated with chronic pancreatitis and a typical regeneration of acinar cells) in males and females. This cirrhosis-like process, not associated with fatty metamorphosis, could be prevented (like the acute lesion) by methionine, suggesting a methionine antagonism as the cause of the liver damage. Bromobenzene is excreted, in part, in the urine as mercapturic acid. This detoxication deprives the body of cysteine and also of methionine, the latter serving as a source of cysteine. In rats intoxicated with bromobenzene, centrolobular hepatic necrosis developed aggravated by preceeding protein depletion. This lesion was prevented by administration of cystine and methionine parallel with an increase of mercapturic acid excretion. Bromobenzenes substituted in the para-position (at which cysteine is coupled) failed to produce necrosis in contrast to derivatives substituted otherwise. This hepatic necrosis represents an effect of depletion of sulfoamino acids. Therefore, examples of hepatic necrosis may be produced by nutritional deficiency, antagonists to and depletors of amino acids.

Morphological and biological changes in mouse ascites tumors following induced infection with certain viruses. IRENA KOPROWSKA AND HILARY KOPROWSKI (introduced by N. CHANDLER FOOT). *Dept. of Anatomy, Cornell Univ. Med. College, New York City, and Sec. of Viral and Rickettsial Research, Lederle Labs. Div., American Cyanamid Co., Pearl River, N. Y.*

The ascites tumors of mice offer many advantages over solid tumors for studying the effect of viral infection upon the neoplastic process. Mice bearing the so-called Ehrlich ascites tumor were injected intraperitoneally with aliquots of mouse brain suspensions infected with 12 neurotropic viruses, respectively. Three to 5 days after the viral inoculation, the abdomens of the animals were tapped and the ascitic fluids withdrawn. Smears were prepared and stained by the Papanicolaou method, and the number of cancer cells were counted. Also, aliquots of uncentrifuged ascitic fluid were bioassayed for tumor cells by subcutaneous route. A portion of ascitic fluid was centrifuged and the supernatant was injected in serial tenfold dilutions intracerebrally in mice to determine the concentration of virus in the infected fluids. The results of these investigations indicated that although the mice succumbed to an encephalitis caused by Eastern equine encephalomyelitis, Western equine encephalomeylitis or Semliki forest viruses, none of these agents parasitized the tumor cells and no effect on the number of cancer cells was observed. In contrast, Bunyamwera virus parasitized the tumor cells in every instance and apparently ultimately destroyed them, for cancer cells previously observed on smears disappeared and the results of bioassay were negative. West Nile virus seemed to interfere with and retard the neoplastic process but apparently had no action on the number of cancer cells.

Antihemophilic factor: its effect on one-stage clotting tests as basis for a simple AHF assay procedure. ROBERT D. LANGDELL,* ROBERT H. WAGNER* AND K. M. BRINKHOUS. *Dept. of Pathology, Univ. of North Carolina, Chapel Hill.*

Hemophilic plasma clots less rapidly than normal plasma with certain thromboplastic agents, including crude cephalin (Mills, 1926), platelet suspensions (Eagle, 1935) and highly diluted active tissue thromboplastins (Dam, 1943). Undiluted tissue thromboplastins (lung and brain) following simple sedimentation in the ultracentrifuge at about 75,000 *g* for 30 minutes act similarly. An abnormally prolonged clotting time with these thromboplastins, and a normal 'prothrombin' time of the same plasma with potent tissue thromboplastins provide strong presumptive evidence of hemophilia. Normal plasma added to hemophilic plasma reduces or eliminates the differential acceleratory action of this group of thromboplastins. The relative inactivity of these thromboplastins in the clotting of hemophilic plasma apparently results from deficient plasma antihemophilic factor (AHF). When less than about 15% of the plasma mixture is normal plasma, clotting times with these thromboplastins, after recalcification, furnish an index of the amount of AHF present. On this basis, a simple one-stage AHF assay has been developed. The antihemophilic activity of an unknown sample can be expressed in percentages (control plasma equals 100%). Both canine and human hemophilic plasmas have been used as substrates. This test has been used for the past 1½ years in studying normal canine and human plasmas, sera with varying AHF content, and plasma fractions. Results with this method show good agreement with those obtained with the Graham AHF assay (*Federation Proc.* 10: 355, 1951). The influence of other coagulants on the specificity of this one-stage method is discussed.

Relation between protein depletion and protein synthesis in albino rat. NORMAN D. LEE* AND ROBERT H. WILLIAMS. *Dept. of Medicine, Univ. of Washington Med. School, Seattle.*

When isotopically labeled amino acids are used to measure body protein synthesis, the extent to which they may be incorporated into proteins is determined by 2 classes of factors, *1*) those concerned with the rate constants for protein synthesis and breakdown and *2*) those concerned

with the dilution of the labeled amino acid by tissue amino acid pools. We have investigated the relation between protein anabolism and protein depletion, using S^{35}-labeled cystine, by controlling dilution factors through dietary means. Groups of albino rats were fed different diets for 7 days. These diets were varied with respect to the quantity and biological value of their protein constituent and with respect to non-protein diets supplemented with inert L-cystine. On the 8th day a standard dose of S^{35}-labeled DL-cystine was administered intraperitoneally and 8 hours later the animals were sacrificed. Liver and plasma proteins were analyzed for radioactivity and sulfur and the extent of labeled cystine incorporation was determined. Increasing the dietary content of a nutritionally adequate protein resulted in diminished cystine-S^{35} incorporation into protien. However, if a nutritionally inadequate protein were substituted, incorporation of the label was increased. If the dietary protein content was very markedly reduced, incorporation was further increased. Comparison of the protein diets with non-protein cystine-supplemented diets indicates these increases not to be the result of amino acid pool changes but of increases in the rate constants for protein synthesis. Our results are consistent with the hypothesis that the rat adapts to protein depletion by increasing its rate of protein synthesis.

Blood volume of excised organs. A. E. LEWIS, R. D. GOODMAN AND E. A. SHUCK (introduced by S. C. MADDEN). *Pathology Sec., Atomic Energy Project, School of Medicine, Univ. of California at Los Angeles.*

In order to distinguish between tissue weight and weight of trapped blood in excised organs of rats, the organ blood volumes were estimated in 2 ways: *1*) the plasma volume was estimated by comparing the amount of T-1824 extracted from a homogenized organ with that in a known volume of blood; *2*) the erythrocyte volume was similarly estimated by comparing extractable hematin from the homogenate with blood. As noted by others, the calculated hematocrit varied in different organs being, except for the spleen, generally lower than the average venous hematocrit. The following mean organ blood volumes (expressed as cc/gm. of wet tissue) were obtained on Wistar strain rats: Kidney, 0.278 (+0.038); liver, 0.178 (±0.012); spleen, 0.481 (±0.060); heart and lungs combined, 0.279 (±0.152). Figures in parentheses give 95% confidence limits of the mean. (Based on work performed under Contract No. AT-04-1-GEN-12 between the Atomic Energy Commission and the University of California at Los Angeles.)

Mechanism of destructive action of Bunyamwera virus on Ehrlich mouse ascites tumor. ROBERT LOVE AND HILARY KOPROWSKI (introduced by G. JOHN BUDDINGH). *Sec. of Viral and Rickettsial Research, Lederle Labs. Div., American Cyanamid Co., Pearl River, N. Y.*

Intraperitoneal inoculation of Bunyamwera virus on the 4th to 5th day after transplantation of Ehrlich ascites tumor results in a rapid destruction of tumor cells. The visible destructive action of the virus begins on the second day after inoculation and within 24 hours only a very occasional tumor cell persists. Within 48 hours no tumor cells remain. The amount of fluid diminishes rapidly and 48–60 hours after virus inoculation no fluid can be obtained. The mice succumb to the virus infection in 5–7 days. Two-hourly observations of the tumor during the destructive phase suggest that 2 mechanisms are involved. The first affects intermitotic cells and consists essentially of an increase in parachromatin associated with some chromatolysis and the appearance of small intranuclear 'inclusions.' The resultant changes in nuclear structure lead to rupture of the nuclear membrane and disintegration of the cell. In addition, many of the cells undergo a type of karyorrhexis which appears to be the result of an abnormal fusion of chromosomes in mitosis. The nature of these phenomena will be discussed.

Dietary glycine nitrogen metabolism as influenced by protein feeding and prior protein fasting, studied with N^{15}. S. C. MADDEN, L. I. GIDEZ* AND J. A. FANCHER, JR.* *Div. of Pathology, Brookhaven National Lab., Upton, N. Y. and Dept. of Pathology, School of Medicine, Univ. of California, Los Angeles.*

Adult dogs fed for more than six months a measured synthetic diet with casein as sole source of protein nitrogen were given 28 mg. glycine labeled with N^{15} per kilogram body weight. Prior to labeling day two of four dogs were protein-fasted for two weeks whereas the other two were fed casein diet. On labeling day one of the protein-fasted pair was fed proteinless diet with labeled glycine, the other casein diet with labeled glycine. Similarly on labeling day of the protein-fed pair one was given proteinless diet with labeled glycine and the other casein diet plus labeled glycine. All dogs were given proteinless diet for the subsequent two weeks. Urine and blood were analyzed by chemical and mass spectrometric methods. During the first days after labeling day the protein-fasted dog which had been fed proteinless diet plus labeled glycine excreted the least N^{15} of the group, half as much (14%) at the end of three days as the protein-fed dog labeled during casein feeding. The other protein-fasted dog, which had been fed casein diet and labeled glycine, excreted the label nearly as copiously as the protein-fed one labeled during casein feeding, whereas the protein-fed dog labeled during protein-fasting had almost as low an excretion rate as the protein-fasted dog labeled

during protein-fasting. These observations will be discussed.

5-Nucleotidase, alkaline phosphatase and hexose-diphosphatase in human arterial walls. J. F. A. McMANUS, C. H. LUPTON, JR.* AND E. M. ETHERIDGE.* *Dept. of Pathology, Univ. of Virginia School of Medicine, Charlottesville.*

The alkaline phosphatase method of Gomori has been used to study arterial walls in human tissues Specific substrates which differ in their histochemical distribution were used. Glycerophosphate has been used as substrate for the alkaline phosphatase or phosphomonesterase I distribution, muscle adenylic acid for the study of 5-nucleotidase and hexose-diphosphate for the hexose-diphosphatase. In the aorta, the alkaline phosphatase method colors the vasa vasorum while the 5-nucleotidase colors the muscular media. 5-Nucleotidase is present in splenic vessels. Smooth muscle appears colored by the 5-nucleotidase in most situations but the coloring of the branches of the renal arteries varies from case to case. In kidneys from normotensive cases, 5-nucleotidase is not seen in the arterial walls. In nearly all situations, the alkaline phosphatase demonstrated with glycerophosphate as substrate is present in vessels only in the endothelium. Hexose-diphosphatase has a distribution partaking of that of the phosphomonesterase I and the 5-nucleotidase. 5-Nucleotidase activity is not seen in the hyaline of arteriosclerosis. It appears in the hayline filling the capsular space in ischemic glomerular obsolescence. Features of distribution in vessel walls as well as influence of pH, the lack of effect of Mg^{++} ion, and the different appearances histochemically in various normal and abnormal structures confirmed the opinion of Reis, Gomori and others that 5-nucleotidase is a specific enzyme.

Chemical and immunological studies on a soluble protein fraction from liver mitochondria. LEO R. MELCHER AND JOSEPH SHACK (introduced by MICHAEL B. SHIMKIN). *Lab. of Exptl. Oncology, Natl. Cancer Inst., Natl. Insts. of Health, Bethesda, Md., and Div. of Medicine, Univ. of California School of Medicine, San Francisco.*

Mitochondira were isolated from guinea pig liver by homogenizing and differentially centrifuging in 0.25 M sucrose according to the method of Schneider and Hogeboom (*J. Biol. Chem.* 183: 123, 1950), modified to process larger quantities of tissue. The mitochondria were suspended in water, the pH adjusted to 7.7 and exposed to alternate freezing and thawing. This suspension was then centrifuged at 20,000 rpm. and the supernatant contained a soluble protein fraction which represented about half of the nitrogen of the mitochondria. Practically all the ribose nucleic acid remained with the insoluble residue. Reproducible electrophoresis patterns were obtained from several such preparations. In pH 8.6 veronal buffer the electrophoretic patterns showed 6 components with mobilities ranging from 2.1–5.4 x 10^{-5} $cm.^2$ $sec.^{-1}$, $volt^{-1}$; no serum albumin was detectable. Zone electrophoresis yielded patterns similar to those found by the moving boundary method. On injection into rabbits both complement fixing and precipitating antibodies were produced. Using this method comparable fractions were isolated from mitochondria of livers of other species. Some of their properties will be presented and discussed.

Pancreatic fibrosis in ducklings as result of nutritional deficiency. O. NEAL MILLER AND R. H. RIGDON. *Dept. of Biochemistry and Nutrition and Lab. of Exptl. Pathology, Univ. of Texas Med. Branch, Galveston.*

Three-day old white Pekin ducklings were placed on a purified diet containing casein, gelatin, celluflour, corn-oil, salts-mixture, corn starch, vitamins A, D, E, K, thiamin, riboflavin, pyridoxine, niacin, pantothenic acid, biotin, and folic acid. When corn starch was replaced by sucrose the birds feathered poorly and developed neurological symptoms characterized by hyperexcitability and leg weakness. After 3–4 weeks on the experimental and control diets no significant difference in growth rate or food consumption was observed. The rate of invertase activity in the duodenum in both groups indicated that 100–125 gm. sucrose could be hydrolyzed/day. Blood fructose level indicated only a slight increase in ducks on a sucrose-diet. Pancreas from ducks eating the sucrose-diet were observed to be hard and white in color but from ducks fed the control diet (corn starch-diet) they were soft, pliable and pink in color. Pathological examination of the pancreas (hard) shows degeneration of acinar cells followed by extensive fibrosis. Islet cells were normal. The incidence of fibrosis in ducks reared on a corn starch- or dextrose-diet was 10–15% as compared to 80–90% in ducks on a sucrose-diet. Addition of B_{12}, inositol, Wilson liver extract, or aureomycin to a sucrose-diet failed to reduce the incidence of fibrosis. Addition of the second mother liquor from crystallization of dextrose reduced the incidence to 20%. Pancreas from ducks raised on natural foods also were free from fibrosis.

Separation, concentration and transfusion of platelets from normal human blood. ALLEN H. MINOR AND LEE BURNETT (introduced by IRVING GRAEF). *Lenox Hill Hosp., New York City.*

About 85% of the platelets in normal human blood may be separated out and concentrated by means of an erythrocyte sedimentation accelerator and a platelet anti-agglutinant, using siliconed

equipment and standard blood bank facilities. Pint volumes of blood of any type, collected by gravity in ACD flasks, are transferred by suction within 3 hours to bottles containing 60 ml. 6% dextrant, intrinsic viscosity 0.39 or higher (Pharmacia), and sedimented at room temperature for about 2 hours. The supernatant suspensions are aspirated into bottles containing 20 ml. 2% Triton WR-1339 (Rohm and Haas), mixed briefly, and centrifuged at 2000 rpm for 30 minutes. All but about 10 ml. of these supernatants are drawn off and discarded, 40 ml. saline added and the platelet-rich sediments resuspended by vigorous shaking. Residual erythrocytes and leukocytes are then removed by brief low-speed centrifugation. The recovered platelets are discrete, morphologically intact and functionally active; 0.1 ml. concentrate added to 3 ml. thrombocytopenic blood induces normal prothrombin consumption and clot retraction. Fourteen transfusions of platelet concentrates, prepared from 4 or 5 pints of blood, have been given to 6 bleeding thrombocytopenic patients: 1 aplastic anemia, 1 acute leukemia and 4 thrombocytopenic purpura. They have been immediately followed by hemostasis, a return to normal of prothrombin consumption, clot retraction, bleeding time and tourniquet test, and variable increases in the platelet count, these effects lasting 1–3 days. There have been no transfusion reactions.

Caffeine gastric analysis in diagnosis of duodenal ulcer. FERRIN B. MORELAND AND DOLPH L. CURB (introduced by BÉLA HALPERT). *Depts. of Biochemistry and Medicine, Baylor Univ. College of Medicine, and Veterans Admin. Hosp., Houston, Tex.*

Sustained hypersecretion of acid by the stomach after caffeine stimulation has been proposed as a diagnostic aid when duodenal ulcer is suspected. Criteria suggested were: 225 mg. free HCl during 90 minutes following stimulation or 50 mEq/l. at 90–120 minutes. The series used in establishing these criteria included patients with proved ulcer and controls presumably free of ulcer. With this type of patient selection, the results of gastric analysis correlated fairly well with the presence of ulcer. To determine whether such correlation would be obtained in patients on whom the test was performed to aid in the diagnosis when the clinical findings were equivocal, a study was made of 28 office patients on whom the test was done because the diagnosis was in doubt. Using the criterion of 225 mg. in 90 minutes, in 2 (25%) of 8 patients with ulcer, the values were lower (false negative) and in 6 (60%) of 10 patients without ulcer the values were higher (false positive); all 4 patients considered as probably having and all 6 considered as probably not having an ulcer secreted over 225 mg. HCl. When the amount or concentration of acid secreted during the last 10 minutes of the 90 minute test period was used, the correlation was even poorer. The results thus indicate that while the test may suggest the tendency to ulcer, it does not add to the results of the clinical observations.

A portable pneumotachograph and its application to a study of human and canine respiration. PAUL E. MORROW (introduced by JAMES K. SCOTT). *Dept. of Radiation Biology, Div. of Pharmacology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

A new pneumotachograph consisting of a low resistance piezometer, a capacitance-modulated high frequency oscillator and a modified FM tuner was constructed. The piezometer and oscillator were designed as a compact portable unit which functions as an electronic manometer. This portable unit permitted studies on ambulatory subjects and in experimental areas where remote measurements were necessary. The manometer and FM tuner units have excellent amplitude and frequency response characteristics below 400 cycles/second. Exemplary of the performance of the pneumotachographic apparatus is the following case: An air-flow through the piezometer of 0.5 l/sec. gives rise to a pressure gradient of less than one ml. of water. This small pressure causes a mechanical change in the variable capacitor of less than 5×10^{-6} inches and in turn creates an 11 kilocycle modulation of the oscillator carrier frequency. The modified FM tuner instrument converts frequency changes to linearly related volts and is independent of signal strength. The 11 kilocycle change produced a voltage that was recorded as a 1.8 inch deflection by a magnetic oscillograph. Respiratory data were obtained from 10 normal human subjects during conditions of rest and stress (air-flow resistances). Canine data were obtained from 5 normal, non-medicated, unrestrained dogs during conditions of rest and exercise. Many dynamic and volumetric measurements were obtained from the pneumotachograms of the human and canine subjects including respired air flow rates, phase and flow durations, and tidal volumes.

Effects of ACTH on wound healing at various levels of ascorbic acid intake. CONRAD L. PIRANI, ROBERT C. STEPTO* AND C. FRANK CONSOLAZIO.* *U. S. Army Med. Nutrition Lab. and Dept. of Pathology, Univ. of Illinois College of Medicine, Chicago.*

Guinea pigs maintained on 'scorbutic,' 'normal' and 'fortified' ascorbic acid intakes (0.0, 2.5 and 25.0 mg/day) were subjected on the 10th day of these regimens to linear laparotomy wounds. Thereafter half of the animals were injected with ACTH (6 mg/day) and half with saline. The ani-

mals were sacrificed in groups on different post-operative days, the wounds studied histologically and the adrenals weighed and analyzed for ascorbic acid and cholesterol content. In the saline group the wounds of the 'scorbutic' animals failed to heal while the 'fortified' guinea pigs, especially on the 5th and 10th postoperative days, showed a better healing than the 'normal' animals. In the ACTH group a moderate inhibition of fibroblastic proliferation and collagen formation was observed in the 'normal' animals on the 5th postoperative day but was less apparent later. ACTH-induced effects were minimal in the wounds of both 'fortified' and 'scorbutic' animals. The results indicate that in the guinea pig high ascorbic acid intake promotes better wound healing. Under these conditions distinct inhibitory effects of ACTH are not observed. The adrenal response to ACTH was somewhat modified by the various levels of ascorbic acid intake, but in general was not prevented. The role of ascorbic acid in wound healing therefore would appear to be a direct one and not mediated through the adrenal cortex.

Protein intake and pulmonary tuberculosis in hamsters and guinea pigs. H. L. RATCLIFFE. *Penrose Research Lab., Zoological Society, and Dept. of Pathology, Univ. of Pennsylvania School of Medicine, Philadelphia.*

Earlier experiments found that the progress of pulmonary tuberculosis in rats and hamsters was accelerated when casein was fed at high levels, and retarded when casein intake was inadequate to maintain normal body weight (*Federation Proc.* 10: 368, 1951). In the present study diets for hamsters contained approximately 3, 10, and 25% animal and vegetable protein. The 25% protein diet, with green vegetables, also was fed to one group of guinea pigs; a second group of guinea pigs received crushed oats and green vegetables. Animals were started on the experimental diets when about 8 weeks old, and 6 weeks later were infected by inhaling small numbers of human tubercle bacilli (H37 Rv strain, Wells' techniques, *Am. J. Hyg.* 47: 1 and 11, 1948). In hamsters killed 75 days after infection, the progress of tuberculosis was unaffected by diet; 125 days after infection the disease was more advanced in the 10% protein group, but thereafter it progressed most rapidly in the 3% protein group. In the 2 groups of guinea pigs tuberculosis developed at about the same rate for 40 days, but 80 days after infection was more advanced in the oats-and-vegetables group. It is concluded that tuberculosis in hamsters and guinea pigs was not influenced by protein intake during its earlier stages, but thereafter its development was modified significantly by the percentage of protein in the diet. Further, the disease in protein-starved animals was retarded briefly, but thereafter its progress was more rapid than in animals on other diets.

Progressive renal disease in subtotally nephrectomized rats. L. J. RATHER. *Dept. of Pathology, Stanford Univ. School of Medicine, San Francisco, Calif.*

Following the removal of 1 kidney and both poles of the other in the albino rat the stump (representing 20–30% of the original two-kidney weight) grows rapidly so that by the 30th post-operative day 55–60% of the predicted 2-kidney weight is present. At this time there is no appreciable renal lesion and the rats are not uremic. During the following months progressive renal disease, characterized by increasing proteinuria, proliferative and sclerotic glomerular lesions, athrocytic phenomena in the tubules, and rising serum creatinine, develops. Elevation of the blood pressure may occur as a relatively late phenomenon. The disease has histopathological features in common with clinical glomerulonephritis, appears to be affected by dietary protein intake, and may have some bearing on the problem of the progression of clinical glomerulonephritis.

Enhanced Nadi reaction in lymphocytes in inflammation. JOHN W. REBUCK. *Dept. of Labs., Henry Ford Hosp., Detroit, Mich.*

Consecutive cover-slip applications, previously described, were utilized to sample the cytology of the exudate at timed intervals throughout the cycle of acute inflammation in man. In control preparations, which were stained like blood smears, the initial migration of neutrophils was supplemented by lymphocytic migrations at 6–12 hours. Lymphocytic hypertrophy began at 12–14 hours and resulted in transformation of lymphocytes into macrophages at 16–24 hours. Next, sites of cytochrome oxidase activity in each of the various cells types were demonstrated separately by the Nadi reaction. Colored photographs and scale drawings were made of the cellular reactions to permit deliberate study of an otherwise transient process. There is general agreement that peripheral blood lymphocytes are relatively deficient in this enzyme system, a finding confirmed by our present study. However by the 12th hour of inflammation, exudative small lymphocytes showed enhanced cytochrome oxidase activity in the form of as many as 60 blue, cytoplasmic granules after treatment with the Nadi reagent. The granules, ordinarily irregularly distributed, were at times concentrated about cytoplasmic vacuoles and occasionally in dense clumps suggesting an origin through lymphocytic ingestion of positively-reacting, neutrophilic, cytoplasmic fragments present in the exudate. Hypertrophied lymphocytes and macrophages were moderately positive. Neu-

trophils were strongly positive at 12 hours. Lymphocytes in this and previous studies were carefully distinguished by marked nuclear and cytoplasmic size-and structure-characteristics from control blood monocytes and control exudative monocytes to be found in lesions produced with parallel high blood monocytoses.

Effect of ribonucleate on globulin production in rabbits undergoing immunization. GOETZ W. RICHTER (introduced by JOHN G. KIDD). *Dept. of Pathology, New York Hosp., Cornell Med. Center, New York City.*

To learn more about the relationship of nucleotides to proteosynthesis, serum proteins were studied by means of the Howe procedure and electrophoresis during 2 months in 4 groups of rabbits: *1*) normal controls; *2*) animals receiving daily subcutaneous injections of sodium ribonucleate; *3*) animals undergoing hyperimmunization with horse serum and receiving daily subcutaneous injections of 0.9% saline; *4*) animals receiving sodium ribonucleate like those of *group 2*, and horse serum like those of *group 3*. The rabbits in *group 1* showed no significant change in globulin levels during the experiment; those of *group 2* generally showed slight increases in beta and gamma globulins, while rabbits of *group 3* developed globulin levels 20–100% above the original concentrations. By contrast, all of the animals in *group 4* manifested globulin concentrations 200–400% above the original levels. In *groups 3* and *4* the increases were principally in the gamma globulin component. Differences between the globulin increments in these 2 groups became apparent within 2 weeks; they were greater after 4 weeks, and generally persisted thereafter though in both groups the globulin levels decreased somewhat after 6–8 weeks. Precipitin tests upon serial dilutions of sera from *groups 3* and *4* disclosed that the antibody levels were comparable in the 2 groups. The findings show that parenteral injections of ribonucleate enhanced the synthesis of serum globulins.

Comparison of bone marrow of normal and anemic mice (*W*-series). ELIZABETH S. RUSSELL AND CORINNE MCLAUGHLIN SNOW (introduced by JACOB FURTH). *Jackson Memorial Lab., Bar Harbor, Me.*

Deleterious genes of the *W*-series cause varying degrees of macrocytic anemia arising before birth and persisting throughout life. Counts of total marrow cellularity from cross-sections of normal and anemic littermates at 0 days and 2, 4, 6, and 8 weeks postnatal showed a constant high level in the normal, severe hypoplasia in the newborn anemics, with increasing cellularity in survivors so that there is little or no difference between genotypes at 4, 6, and 8 weeks. Smears of femoral marrow of normal and anemic littermates at 2, 4, 6, and 8 weeks postnatal, however, showed a persisting qualitative difference. At 2 weeks only the E/M ratios of anemics are low; at all ages there is a deficiency of late stages of haematopoiesis. All of these findings are compatible with a continual maturation arrest probably due to genetic deficiency of a particular essential substance.

Effect of ascorbic acid, folic acid and vitamin B_{12} upon tyrosine oxidation in acetone powder preparations of guinea pig liver. BERNARD SCHEPARTZ AND ELI M. NADEL (introduced by HAROLD L. STEWART). *Dept. of Biochemistry, Jefferson Med. College, Philadelphia, Pa., and Pathology Sec., Natl. Cancer Inst., Bethesda, Md.*

Acetone powder preparations of guinea pig livers when supplemented with α ketoglutarate were found capable of converting l-tyrosine to acetoacetate. As previously reported for powders prepared from rat livers, the catabolism of tyrosine required an initial transamination, with α ketoglutarate as the acceptor of the amino group (B. SCHEPARTZ. *J. Biol. Chem.* 193: 293, 1951). Enzymatic activity was reduced 50% in livers of scorbutic animals. This deficiency was remedied by the *in vitro* addition of ascorbic acid. Decreased food consumption by scorbutic animals was not the cause of the decline in enzymatic activity, for acetone powders prepared from livers of fasted animals that had received oral supplements of ascorbic acid had normal levels of activity. *In vitro* additions of folic acid or of vitamin B_{12} did not restore the activity of preparations from scorbutic animals. Aging of the acetone powders prepared from normal livers caused a decrease in the activity of these powders to levels obtained in preparations from scorbutic animals. Normal activity was restored *in vitro* supplementation with ascorbic acid. The data confirm the reports of other laboratories on the importance of ascorbic acid in tyrosine metabolism.

Hypervitaminosis D in frog, with special reference to changes in paravertebral lime sacs. HANS G. SCHLUMBERGER AND DONALD H. BURK.* *Dept. of Pathology, Ohio State Univ. College of Medicine, Columbus.*

The lime sacs of the frog surround the spinal nerve roots; they are lined by a cuboidal epithelium and filled with a milky suspension of calcium carbonate and phosphate. The sacs are variable in size and become larger and more opaque if the frogs are kept in water containing 0.8% $CaCl_2$. Administration of a single intramuscular injection of vitamin D_2 greatly increases the size and density of the lime sacs as observed in serial

roentgenograms over a 2–3 week period. The blood calcium level rises and that of phosphorus falls. To produce hypervitaminosis D a total of 150,000–300,000 units of vitamin D_2 (activated ergosterol in peanut oil) were given as intraperitoneal or intramuscular injections of 50,000 units weekly. During the experiment the frogs were not fed. The line sacs appeared in the roentgenograms as dense, massive, paravertebral structures 3–4 mm in width extending the entire length of the spinal column. Osteoporosis was characterized histologically by thinning of the bone cortex and focal bone destruction by osteoclasts. In healing fractures calcification of the cartilagenous callus and periosteal bone formation were retarded. When frogs were kept in 0.8% $CaCl_2$ these bone changes were reduced or absent. Metastatic calcification occurred only in the kidney. Intramuscular injection often led to massive fat emboli in kidneys and lungs, associated with calcification in the former but not in the latter. No consistent structural changes were found in the parathyroids.

A spectrophotometric method for studying reactions of lymphocytes to x-rays and other reagents in vitro. ROBERT SCHREK (introduced by WILLIAM B. WARTMAN). *Tumor Research Lab., Research Service, Veterans Admin. Hosp., Hines, Ill.*

Cellular suspensions from the rabbit thymus were irradiated with x-rays (20–1000r) and incubated at 37°C. for 20 hours. According to the method of unstained cell counts, the lymphocytes in the irradiated, incubated suspensions were dead with pyknotic nuclei but most of the cells in non-irradiated, incubated suspensions were viable. Addition of hypertonic saline to the suspensions caused crenation of the viable cells and partial lysis of the pyknotic lymphocytes. As a result, the irradiated suspension with hypertonic saline became less turbid than the non-irradiated control. On spectrophotometric examination, the visibly clear, centrifuged supernatant from the irradiated suspensions showed a greater amount of absorption of ultraviolet light than the non-irradiated control. These findings suggest that the hypertonic saline extracted nucleoproteins from the dead irradiated lymphocytes but not from the viable, non-irradiated cells. An attempt is being made to adapt this spectrophotometric method to the titration of the effect of x-rays and adrenal cortex hormones on lymphocytes.

Continuous measurement of blood dye concentration in intact animal. ELFRED M. SHIDELER* AND HOWARD C. HOPPS. *Dept. of Pathology, Univ. of Oklahoma School of Medicine, Oklahoma City.*

Trypan blue is selectively localized in the reticulo-endothelial system and, therefore, its rate of disappearance from the circulating blood was proposed by us as a measure of reticulo-endothelial function. Determination of blood concentration by means of repeated blood samples is not feasible. We have developed an apparatus for continuous measurement of trypan blue in the peripheral blood of the intact animal. An oscillograph measures light of first one wave length and then another transmitted through a single area in the ear. This is accomplished by a rotating disk which introduces into the light path 2 filters, alternatingly, 15 times each second. Light transmitted through one filter indicates hemoglobin concentration. Light transmitted through the second filter indicates the concentration of hemoglobin plus trypan blue. This allows correction for variation in light transmission because of altered blood flow. In each instance measurement of hemoglobin is independent of oxygen saturation. This apparatus is described and results obtained in rabbits are presented.

Quantitative studies of skeletal effects of high-fat diets. MARTIN SILBERBERG, RUTH SILBERBERG AND MARION OPDYKE.* *Snodgras Lab., City Hosp. Div., and Dept. of Pathology, Washington Univ., St. Louis, Mo.*

In male mice of strain C57 black, a high-fat diet fed *ad libitum* accelerated skeletal aging. In order to test the specificity of the changes observed, quantitative studies were undertaken. Seventy mice of strain C57 received from weaning to 6 months of age diets composed of a common base and isocaloric supplements high in protein, fat, or carbohydrate. Of groups of 5, 1 animal each received 1 of these diets *ad libitum*, and 1 each received the high-fat or high-carbohydrate diet restricted to the *ad libitum* intake of the 'pilot' (high-protein) animal. For each individual, the daily food consumption was recorded; weights were taken once a week. In the growth zones of the long bones, the progress of epiphyseal aging was studied microscopically. Of the animals fed *ad libitum*, skeletal aging proceeded most slowly in the individuals consuming the high-protein diet; skeletal aging was most rapid in animals fed the high-fat diet, while those consuming the high-carbohydrate diet took an intermediate position. In mice fed the high-fat or high-carbohydrate diets in amounts restricted to the caloric intake of the pilot animal, skeletal aging was more rapid in individuals receiving the fat diet, while both groups showed a slower rate of skeletal aging than animals fed the respective diets *ad libitum*. It is concluded that both caloric intake and fat specifically influence the rate of skeletal development and aging. (Aided by grants from the Natl. Insts. of Health and the American Cancer Society.)

Degenerative joint disease in Syrian hamsters. RUTH SILBERBERG, JOHN SAXTON, GLADYS SPERLING* AND CLIVE MCCAY. *Snodgras Lab., St. Louis City Hosp., Dept. of Pathology, Washington Univ., St. Louis, Mo., and Dept. of Animal Husbandry, Cornell Univ., Ithaca, N. Y.*

The kneejoints of 15 male and 20 female hamsters, 637–1148 days old, were studied microscopically. In 5 animals, the articular surfaces were unchanged; in 6, there were slight age changes, such as slight proliferation and hypertrophy of the cartilage with occasional pyknosis of nuclei, in 11 advanced age changes consisting of liquefaction or fraying of the matrix; in 13, there were osteoarthritic lesions characterized by marked growth processes and regressive changes causing ulcers of the articular surface with eburnated osseous floors and hypertrophic margins.

TYPE CHANGE	% TOTAL	AGE (DAYS)	MEAN
No change	14.4	637–887	745
Age change slight	17.1	716–1065	850
Age change advanced	31.4	702–1148	872
Osteoarthritis	37.1	720–1091	811

Although there was a suggested positive correlation of the severity of the age changes with increasing age, the mean age of the animals with osteoarthritis was lower than that of animals with advanced age changes. Aging is thus not the only factor operative in the development of osteoarthritis. While these changes will have to be studied further regarding distribution and etiology, they may be considered, as are the corresponding lesions in the mouse (*Am. J. Anat.* 68: 69, 1941; *Arch. Pathol.* 50: 828, 1950), as an analogue of human osteoarthritis. (Aided by grants from the U. S. Public Health Service.)

Experimental determination of rate of longitudinal bone growth. H. A. SISSONS (introduced by WILLIAM B. WARTMAN). *Dept. of Pathology, Northwestern Univ. Med. School, Chicago, Ill., and Inst. of Orthopedics, London, England.*

By inserting a metal 'marker' into the shaft of a long bone of an animal and taking a series of x ray pictures at intervals during growth, the rate of growth of an individual epiphyseal plate can be determined. Using rats and rabbits, measurements were carried out on the epiphyseal plate of the lower end of the femur. This was found to grow at an average rate of 200 μ/day in the rat, and 350μ/day in the rabbit, during the period of active growth. Data are presented on the retardation of epiphyseal growth produced by the administration of cortisone, and by a series of carcinogenic hydrocarbons and related compounds. The histological structure of the growing zone of the epiphyseal plate in both the normal and the retarded animals is discussed in the light of the measurements of growth rate.

Stress reactions and tumor-breaking property of bacterial polysaccharide. BORIS SOKOLOFF, WALTER H. EDDY, GEORGE CONE AND RITA POWELLA (introduced by LEO LOEB). *Southern Bio-Research Lab., Florida Southern College, Lakeland.*

Diller *et al.* suggested that the alarm reactions induced by bacterial polysaccharide caused hemorrhages in the tumor and the adrenal gland. One of the chief features of the alarm reactions is increased capillary fragility. To prove that the tumor-breaking property is related to increased capillary fragility, Crocker Rat Carcinoma, average size 7–8 cm.^{3} was used. In the first series, 25 rats received simultaneously 0.5 mg/100 gm./wt. of P-25, Shear bacterial polysaccharide, and 10 mg 100 gm./wt. of flavonoid compound. 66% survived, the rest lived, on the average, 46 hours. The controls receiving 0.5 mg/100 gm. wt. of P-25 succumbed in 7 hours 44 minutes (average). Flavonoids prevented the increase in capillary fragility. In the next series, 25 rats, bearers of Crocker Carcinoma were placed on a Sherman-LaMer scorbutogenic diet to which 5% D-gluco-ascorbic acid was added. 5 days later the D-gluco-ascorbic acid was reduced to 3%. The ascorbic acid level of blood plasma was brought down to 0.01 mg:100 cc. plasma and 0.1 mg:100 gm. wt. of P-25 was then injected. Mortality rate was 25% against 15% of the control group kept on regular diet, but the area of tumor-destruction was 5.3 times larger than in control animals. These results indicate that increased capillary fragility is the prime factor in tumor-breaking property of bacterial polysaccharide.

Effect of ascorbic acid analogue on radiosensitivity of transplanted tumors. BORIS SOKOLOFF, WALTER H. EDDY, LEO STRELTZOV AND GEORGE CONE (introduced by LEO LOEB). *Southern Bio-Research Lab., Florida Southern College, Lakeland.*

An almost complete withdrawal of ascorbic acid from the system increases the radiosensitivity of Crocker Rat Carcinoma. 3000 or 5000 r contact radiation applied to 18 mm.^{3} size carcinoma of rats on regular diet produces only a partial destruction of the tumor and prolongs the span of life of the animals, on an average, 48 days after 3000 r, and 57 days after 5000 r. Sixty rats were placed on a Sherman-LaMer scorbutogenic diet to which 5% D-fluco-ascorbic acid and 0.5% flavonoids were added. Five days later, D-gluco-ascorbic acid was reduced to 3%. When the size of tumor reached 19–20 mm.^{3}, 30 rats were submitted

to 3000 r contact radiation in a single dose and 30 rats to 5000 r. All tumors were completely destroyed and all rats except one survived. Radiation factors were the same in all experiments: Chaoul contact apparatus, 60 KV, 0.12 mm.^{3} filter, 3 cm. distance from the target, field 2 cm. diameter with 450/min. Ascorbic acid content of plasma at time of radiation was 0.025 mg/100 cc. of plasma (average). These experiments indicate that a temporary but almost complete withdrawal of ascorbic acid from the system results in the same complete destruction of Crocker Carcinoma with smaller doses of radiation (3000 r and 5000 r) as is achieved with much larger doses (8000 r and 10000 r) when the ascorbic acid level is normal (0.4 mg/100 cc. of blood plasma).

Effect of vitamin C-free diet on ascorbic acid content and white blood cells of Ak leukemic mice. Boris Sokoloff, Walter H. Eddy, John Beaumont, Rita Powella and George Cone (introduced by Leo Loeb). *Southern Bio-Research Lab., Florida Southern College, Lakeland.*

It is known that the ascorbic acid content of leukemic wbc is higher than that of normal mice. One hundred Ak mice showing symptoms of leukemia were divided into 2 groups. Fifty mice were placed on a Sherman-LaMer diet to which 5% D-gluco-ascorbic acid and 0.5% flavonoids were added. Five days later, D-gluco-ascorbic acid was reduced to 3% and 5 days afterward to 2%. Mortality rate was 6%. The ascorbic acid content was reduced to 0.05 mg/100 cc. plasma and maintained at this level. The number of white blood cells dropped, but more slowly, and reached a subnormal level of 2,500 (average). This level was maintained for several weeks without apparent ill effect. Another group was submitted to the identical regime except that no flavonoid was given. Mortality rate: 65%. Animals manifested tendency to bleed. Ascorbic acid content was maintained at 0.05 mg/100 cc. plasma level and number of white blood cells at 2,800 (average). The flavonoids minimized the ill effect of scorbutogenic diet and permitted maintenance of white blood cells at a subnormal level. The results of this investigation indicate that the analogue of ascorbic acid, D-gluco-ascorbic acid, interferes with the synthesis of this factor.

Impairment of antimicrobial defenses following total body irradiation of mice. R. S. Speck, E. Jawetz, and H. S. Kaplan (introduced by Alvin J. Cox, Jr.). *Div. of Microbiology, Univ. of California School of Medicine, and Dept. of Radiology, Stanford Univ. School of Medicine, San Francisco.*

Infection plays an important part in the death of experimental animals following systemic x-irradiation. Bacteria gain access to the body through denudation of epithelium, but other mechanisms which ordinarily remove such microorganisms promptly must also be impaired. Experiments were designed to elucidate the nature of this defect. A standard subacute infection was induced by injecting 100 LD_{50} streptococcus C 203 intramuscularly into 15 gm. C_{57} black mice 1–3 days after a single whole body dose of 450 r, and into non-irradiated litter-mate controls. Two types of observation were made: the rate of death, and the rate of spread of infection as indicated by semiquantitative organ cultures from serially sacrificed animals. In the controls this infection progressed from the site of injection via the lymphatics to reach the blood stream in 2–3 days. The filtering action of spleen and liver removed most of the bacteria for 2–3 more days, delaying overwhelming bacteremia and death until 5–7 days after inoculation. In irradiated animals the streptococci disseminate more rapidly and the animals succumb 3–4 days earlier than the controls. Cultures indicate that both lymphatic blockage and the filtering action of spleen and liver are virtually ineffective after irradiation. Penicillin (200 units daily/mouse for 3 days) completely eliminated the infecting microorganisms and prevented deaths in the controls. In irradiated mice penicillin eliminated bacteria, except at the local site of infection, and reduced mortality to the level of non-infected irradiated mice. The local lesion in irradiated animals exhibited liquefaction necrosis, perhaps interfering with access of the antibiotic, and a striking dearth of inflammatory cells, permitting persistence of microorganisms.

Mechanism of leukocytic responses to provocatives. Bernhard Steinberg and Ruth A. Martin.* *Toledo Hosp. Inst. of Med. Research, Toledo, Ohio.*

Many investigators have noted a leukopenia frequently followed by leukocytosis after injection of certain substances. Several explanations were advanced to account for these changes: increased stickiness of leukocytes and adhesion to vascular endothelium (Essex and Grana. *Am. J. Physiol.* 158: 396, 1949); redistribution within vascular channels (Kemp, Cartwright and Wintrobe. *Am. J. Physiol.* 165: 554, 1951); presence of a leukopenic factor in leukocytes (Weisberger, Heinle and Hannah. *Proc. Soc. Exper. Biol. & Med.* 70: 749, 1949); and action of a factor from inflammatory area (Menkin. *Newer Concepts of Inflammation.* Springfield, Ill.: Thomas, 1950). Steinberg and Martin (*Am. J. Physiol.* 161: 14, 1950) disregarded the primary leukopenia as nonspecific and described in human plasma an expulsion factor which delivers leukocytes from bone marrow to circulation to replace those cells destroyed in the normal process of

aging and disintegration. In this study, the total leukocytic response to milk and other provocative substances was investigated. Peripheral blood, bone marrow and other viscera were studied in normal animals and in those deprived of spleen, adrenals, or both of the organs. Two distinct mechanisms could be recognized, one nonspecific, which did not involve the marrow, and the other specific in which marrow and peripheral leukocyte content were interelated. Confusion of the 2 responses hinders interpretation of the effects on the hemopoietic system of investigative procedures. These studies also suggest that certain types of leukopenia may be accounted for by an increased rate of cell destruction.

ACTH content of pituitary at different levels of ascorbic acid intake. ROBERT C. STEPTO,* CONRAD L. PIRANI, JOSEPH D. FISHER* AND KENNETH SUTHERLAND.* *U. S. Army Med. Nutrition Lab., and Armour Labs., Chicago, Ill.*

The pituitary glands of guinea pigs kept on different levels of ascorbic acid intake (0.0, 2.5, and 25.0 mg/day for 14 and 21 days, and 0.2 mg/day for 6 weeks) and of their pair-fed controls were assayed for ACTH content and examined histologically. After 14 and 21 days on the experimental regimen the ACTH activity of the guinea pig pituitaries was: deficient animals (0.0 mg. intake), 54.0 and 89.5 gamma, respectively; normal animals (2.5 mg. intake), 72.0 and 70.7 gamma; and fortified animals (25.0 mg. intake), 63.0 and 87.0 gamma. Pituitary glands of animals kept on a chronic depletion regimen for 6 weeks (0.2 mg. intake) had the following ACTH activity values: deficient, 130.8 gamma; normal, 64.5 gamma; and fortified, 86.2 gamma. The average ratio of pituitary ascorbic acid to ACTH content in the normal animal was 1:38 (gamma/gamma) as compared with 1:17 in the fortified animals. This ratio in the deficient animals showed a progressive increase from 1:50 at *day 14* to 1:62 at *day 21* to 1:110 at 6 weeks. This increase was not observed in the pair-fed controls. The results indicate a rapid increase in pituitary ACTH content as the duration of ascorbic acid depletion increases. Histologically an increased basophilism was observed in the deficient animals. These data and previous studies from our laboratory suggest a relationship between ascorbic acid intake and ACTH activity of the pituitary.

Fat nephrosis produced by colchicine. STEPHEN S. STERNBERG AND FRANK C. FERGUSON (introduced by FRED W. STEWART). *Pharmacology Sec., Sloan-Kettering Inst. for Cancer Research, and Dept. of Pharmacology, Cornell Med. College, New York City.*

Toxic doses of colchicine produce renal changes, not previously recognized, in both rats and cats. The pathological change observed in the kidney is termed 'fat nephrosis' as defined by Allen (*The Kidney. Medical and Surgical Diseases.* New York: Grune and Stratton, 1951). A variety of agents (including phosphorus, carbon tetrachloride and iodoform) may produce an identical picture in humans and animals. With all these poisons (including colchicine), there develops throughout the proximal nephron cytoplasmic deposition of non-anisotropic fat with basalar vacuolization and nuclear elevation. (The term 'lipoid nephrosis' is reserved for conditions associated with the nephrotic syndrome and seen principally with certain forms of glomerulonephritis, in which fat is deposited in the proximal nephron in a diffuse lattice-work rather than basalar arrangement; anisotropism is usually seen, and deposition may occur not only interstitially but even in the glomeruli.) In rats, the fat nephrosis was found in its classical form in animals sacrificed 17 hours after a single intravenous dose (4 mg/kg.). In chronic toxicity experiments the lesion was incompletely formed, with fat deposited irregularly and in lesser amounts. In cats, however, the lesion was full-blown in both acutely and chronically intoxicated animals. The nephrosis was seen within 24 hours following a single intravenous dose of 0.5 mg/kg. and also after 25 days of repeated small doses totaling 0.8 mg/kg.

Observations on experimental testicular tumors in rats. WILLIAM H. STERNBERG,* RICHARD COPPEDGE* AND ALBERT SEGALOFF. *Depts. of Pathology and Medicine, Tulane Univ., and Ochsner Fndn., New Orleans, La.*

Testicular tumors were observed in the spleens of 17 of 22 castrated, adult AxC rats, 7–31 months following intrasplenic implantation of whole testis from 1- or 2-day old donors. Autogenous transplant of testicular fragments into the spleens of 18 45-day old castrate rats failed to produce tumors. Intrasplenic testicular transplants examined before tumor development had occurred revealed hyperplastic Leydig cells and tubules containing Sertoli cells only. The intrasplenic tumors showed variegated tissue patterns, including well-differentiated Leydig-cell tumors, tubular structures lacking spermatogenic elements, and lipid-rich cells growing diffusely and in pseudotubular formations. The latter cells were occasionally present within surviving testicular tubules, and in rare instances showed evidence of extension through the tubular wall into surrounding stroma. Although these cells bear some resemblance to Leydig cells, they are probably neoplastic Sertoli cells. A few tumors contained areas of small undifferentiated cells. Seminoma, embryonal carcinoma and teratoma were not observed. Transplants of the intrasplenic tumors into castrate hosts were successful in the spleen,

but not in the subcutaneous tissues. One rat, with a large primary intrasplenic tumor, had seminal vesicle enlargement probably indicative of androgen production by the tumor. In a separate series, 2 of 8 rats rendered cryptorchid had small tumors of pure Leydig cell type in the testis 2 years after operation. Seven non-testicular neoplasms occurred in 6 of the 8 cryptorchid animals, including tumors of prostate, penis, salivary gland and forestomach.

Resistance to grafting with lymphosarcoma cells following injection of intact, homologous lymphoid cells. H. C. STOERK, TATIANA BUDZILOVICH* AND T. C. BIELINSKI.* *Merck Inst. for Therapeutic Research, Rahway, N. J.*

Recently it was observed that suspension of intact lymphoid cells of mice or rats in which tumor transplants had regressed, and which subsequently had become resistant to reinoculation with tumor tissue, were cytotoxic to the respective neoplastic cells; lymphoid cells from normal animals showed no such activity. However, when attempts were made to passively transfer 'tumor immunity' it was found that a single injection of intact lymphoid cells to susceptible rats, after a delay of 2 days, caused resistance to grafting with lymphosarcoma cells irrespective of whether the lymphoid cells were derived from tumor-immune or from normal rats. This resistance lasted for about 3 weeks. It was limited and was relative to the number of cells in the inoculum. The resistance to tumor grafts developed only with uninjured, homologous lymphoid cells. Injection of suspensions of intact heterologous or autologous lymphoid cells did not alter the susceptibility of the animals. Furthermore it was seen that the acquired resistance to grafting with lymphosarcoma cells was lost when, following the injection of homologous lymphoid tissue, cortisone was administered prior to the challenging inoculation. Although other possibilities cannot be excluded, it appears likely that some form of immunity related to iso-antigens explains the present observations.

Fate of intravenous C^{14} labeled Dextran in dogs. ROGER TERRY AND CHARLES L. YUILE. *Dept. of Pathology, Univ. of Rochester, School of Medicine and Dentistry, Rochester, N. Y.*

Four normal mongrel dogs received Dextran labeled with Carbon 14. The radioactivity was measured in the urine, plasma, and expired air. The latter was collected in hourly samples at various times. The experiments were terminated 3, 5, 12, and 14 days after the Dextran was injected intravenously. The last 3 dogs were killed and measurable radioactivity was found in various tissues. The molecular size of the administered Dextran is not accurately known. The dogs excreted 57–72% of the injected dose into the urine in 24 hours. Within the first 3 days after injection the levels of Dextran in the plasma fell to almost negligible amounts. The total C^{14} collected from the expired air varied from 4.4–20% of the injected dose in the shortest to the longest experiment. The rate of $C^{14}O_2$ excretion reached a high point during the first day and then fell off fairly steadily to the end of each experiment. None of the animals showed any evidence of toxicity to the material administered.

Factors which influence phagocytosis by reticuloendothelial cells in early hours of inflammation in subcutaneous areas. EDNA H. TOMPKINS. *Cancer Research Inst., New England Deaconess Hosp., Boston, Mass.*

Phagocytic activity of reticulo-endothelial cells was investigated in the subcutaneous connective tissue of mice by use of: *1*) a birefringent lipid, *2*) India ink, *3*) bacteria and *4*) erythrocytes. These were suspended in serum or glucose, and injected subcutaneously (*in vivo* studies) or used to cover living films from areas injected 2–4 hours previously with glucose or mixtures with serum (*in vitro* studies). The control experiments consist of similar studies on areas of the same mice injected shortly enough before sacrifice to exclude influx of polymorphonuclears. The films were studied supravitally and after fixation. Phagocytosis was judged by the presence of the experimental substances in, or on, the phagocytes. Polymorphonuclears were the only hematogenous cells involved, since the experiments terminated within 5 hours of the injections. Reticulo-endothelial phagocytosis is, therefore, represented by preexistent mononuclears only, that is, by the tissue clasmatocytes called 'resting-wandering' cells. The findings demonstrate that: *1*) phagocytosis and removal of particulate substances by reticuloendothelial cells of connective tissues begins in the first hours of inflammation while the 'resting-wandering' cells are still fixed; *2*) this is influenced by the length of time for exposure and the particulate size of the substances presented for phagocytosis; *3*) the cells are not chemotactic and particles must be brought into direct contact with them; and *4*) the latter is aided by muscular movements, by amoeboid activity of polymorphonuclears, whether or not they themselves are phagocytic of the particulate substances, and by the gradual cytoplasmic changes in tissue clasmatocytes incident to subcutaneous injection of any fluid. (Supported by AEC Contract AT (30-1)-901.)

A transmissible disease in mice characterized by anemia, leukopenia and erythropoietic splenomegaly. A. C. UPTON* AND J. FURTH. *Biology Div., Oak Ridge Natl. Lab., Oak Ridge, Tenn.*

A disease characterized by anemia, leukopenia, and extensive erythropoiesis in the spleen, with splenomegaly, has been transmitted in 20 passages (6 subpassages). This disease was encountered in a female mouse and transmitted by intraperitoneal injections of particles of involved spleens. The interval between inoculation and appearance of symptoms has been constantly about 3–5 months. The course of the disease has been typically chronic, the splenomegaly and anemia progressing gradually for several months prior to the death of the animal. The size of the spleen usually exceeds 35 x 15 mm. in the 2 greatest dimensions, the enlargement being due principally to erythropoiesis. Leukemic infiltrations are lacking, and hemopoiesis in the bone marrow does not appear to be disturbed. Some blood destruction is indicated by hemosiderosis and erythrophagocytosis in macrophages of spleen, lymph nodes, and bone marrow. There is erythroblastosis and reticulocytosis of the peripheral blood, and the erythrocyte counts drop below 1,000,000. Thus far no organisms have been demonstrated in histologic sections or blood films. Attempts are being made currently to establish the nature and causative agents of this disease.

Structure and function of cardiac conduction system of dog. GORDON F. VAWTER,* C. BRUCE TAYLOR,* OGLESBY PAUL* AND GEORGE M. HASS. *Rush Lab. of Pathology, Presbyterian Hosp., and Aero-Med. and Physical Environment Lab., Univ. of Illinois, Chicago.*

Hearts of 41 nembutalized dogs were exposed surgically. Lesions along the cardiac conduction system were produced with a special freezing instrument. All frozen cellular structures became non-viable but the persistence of extra-cellular structure preserved anatomic continuity. Electrocardiograms were made before, during and at intervals up to 7 days after operation. After death or sacrifice the coronary arteries of damaged hearts were immediately perfused with cold absolute alcohol. Microscopic studies were made of serial sections stained for glycogen from tissue blocks which included lesions, adjacent tissue and structures of the conduction system. Discrete focal lesions properly placed were accompanied by permanent atrio-ventricular block in 3 animals, right bundle branch block in 3 animals and left bundle branch block in 3 animals. These lesions involved glycogen-rich myofibers of the main bundle of His, the right bundle branch and the left bundle branch of His, respectively. Lesions which only partially involved or were close to the glycogen-rich bundles were not accompanied by permanent conduction disturbances. Only temporary atrio-ventricular block or nodal rhythm occurred in 8 animals, when lesions were made in the theoretical location of the atrio-ventricular node. Temporary or permanent changes in the auricular pace maker in 8 animals followed lesions along the sulcus terminalis. Other electrocardiographic patterns occurred temporarily. These were attributed to manipulation.

Choline absorption from small intestine of rat. JOHN W. VETPER,* JESUS DE LA HUERGA,* MORTON I. GROSSMAN* AND HANS POPPER. *U. S. Army Med. Nutrition Lab. and Hektoen Inst. for Med. Research, Chicago, Ill.*

The following technique was divided to study disappearance of choline from the small intestine of the normal rat and rats with hepatic injury. The animals were fasted for 48 hours prior to the experiment and, under ether anesthesia, the small intestine was ligated just below the entrance of the common bile duct and just above the ileocecal valve. A 2.0 ml. test solution was instilled through a cannula just below the upper ligature, and the intestine again ligated below the puncture site. The abdominal wall was closed and the animal was kept fasting for a specified time, after which it was sacrificed and the small intestine removed *in toto*. The lower end was then cut away and the contents were milked into a test tube. Ten ml. normal saline wash was run through by means of a cannula at the upper end, and the residual was milked through. The contents and washing were analyzed for choline content. Controls, in whose intestines normal saline solution containing 20 mg. choline base as choline chloride was instilled, demonstrated mean choline disappearances of 33.46, 40.57, and 82.07% in 1, 2, and 4 hours. Another series received 20,000 units of an insoluble penicillin in addition to the choline. Similar studies were performed in which *a*) animals given 1 mg. ethionine/gm. body weight as an hepatotoxin intraperitoneally 48 hours before the experiment, *b*) animals fed a high carbohydrate diet containing 0.5% ethionine for 4 weeks, were used. Comparative disappearance data will be presented.

Opsonins for rickettsiae. JOSEPH VICTOR, RUTH RAYMOND,* JEANNE VALLIANT,* JOHN C. WAGNER* AND ABOU D. POLLACK.* *Chemical Corps Biological Lab., Camp Detrick, Frederick, Md.*

A method has been developed for the quantitative determination of blood opsonins for *Coxiella burnetii*. An opsonin unit for *C. burnetii* is the quantity of opsonin causing phagocytosis of rickettsiae by 94–100% of polymorphonuclear neutrophiles under standard conditions. The opsonin titer is the number of opsonin units in 0.1 ml. serum or plasma. An opsonin titer of 1 occurred in 1% of individuals having no known contact with *C. burnetii*; in 30% of individuals previously vaccinated with chick embryo grown *C. burnetii*

and in 31% of normal individuals were Q fever is; endemic. Statistically, about 42% of people in the latter 2 groups showed increased opsonin for *C. burnetii*. Where Q fever is endemic, positive complement-fixing antibodies occurred only in 1/7 of individuals with elevated opsonins. In contrast, vaccinated individuals showed no relation between complement fixation and opsonin titers. Injection of normal chick embryo yolk sac increased opsonins for rickettsiae grown in yolk sac. This may explain the different serological reactions of artificially and naturally immunized populations. In patients with Q fever, opsonin titers increased many million fold, often 10,000 times more than complement fixation titers. This opsonin test may be used as an epidemiological and diagnostic method for detecting infection with *C. burnetii*.

Effect of absorption upon carcinogenicity of hydrocarbons. E. VON HAAM, H. L. TITUS,* GEORGE Y. SHINOWARA* AND I. T. CAPLAN. *Dept. of Pathology, Ohio State Univ., Columbus.*

In previous studies von Haam and Mallette showed that benzene extracts of industrial carbon blacks possess a certain degree of carcinogenicity. Ingalls, on the basis of his clinical investigations, stated that "The carbon black worker faces no worse than the ordinary risk of cancer encountered by other groups of the male population . . ." For this reason the capacity of carbon blacks to absorb and thus render ineffective carcinogenic hydrocarbons was investigated. 3,4-Benzepyrene, 20-methylcholanthrene and *p*-dimethylaminoazobenzene were chosen as effective carcinogenic hydrocarbons which in our laboratory produced skin epitheliomas, fibrosarcomas, and hepatomas in over 50% of our positive control groups within 12 months. Eight carbon blacks differing in acidity, particle size (electron microscope value) and total surface served as adsorbents; cyclohexane, acetone and olive oil were used as organic solvents. In a series of chemical tests the saturation factor of the various carbon blacks for each hydrocarbon was determined and samples of carbon blacks were prepared fully saturated with the various hydrocarbons. Biological experiments with those absorbed hydrocarbons revealed a marked loss of the carcinogenic properties which were in direct proportion to the absorptive power of various carbon blacks.

Histochemical demonstration of 5-nucleotidase activity in cell nuclei. M. WACHSTEIN AND E. MEISEL.* *Dept. of Pathology, St. Catherine's Hosp., Brooklyn, N. Y.*

Evidence has been brought forward recently which seriously questions the value of histochemical methods for the localization of alkaline and acid phosphatase within cell nuclei. While these enzymes were found predominantly in the cytoplasm, large amounts of 5-nucleotidase were present in the nuclei of rat liver as determined chemically in fractionation studies (NOVIKOFF, PODBER AND RYAN, *Federation Proc.* 9: 210, 1950). The histochemical demonstration of this enzyme in cell nuclei in tissue sections under controlled conditions is reported. Myometrium of human uterus and rabbit aorta were chosen as test material because they did not show any unspecific alkaline phosphatase activity in cell nuclei even after many hours incubation with glycerophosphate as substrate. The occurrence of nuclear 5-nucleotidase in other organs will also be presented. With myometrium from human uterus and rabbit aorta not only cytoplasmic but nuclear activity could be demonstrated using adenosine-5-phosphoric acid as substrate in the Gomori-Takamatsu technique. It became demonstrable within 3–6 minutes incubation at 37°C. in paraffin sections of acetone-fixed tissues as well as in frozen sections of fresh or acetone-fixed material. The sites of enzymatic activity were identical in all 3 preparations. Paraffin sections incubated for 10–60 minutes were well suited for microscopic study. Optimal activity was obtained between a pH of 7.5–8.2. The addition of magnesium sulphate to the substrate mixture led to only slight activation of the enzymatic staining. Potassium cyanide inhibited the histochemical staining reaction if magnesium was omitted from the incubation mixture but did not completely suppress it in the presence of magnesium. No unspecific staining due to absorption of liberated calcium phosphate or diffused enzymes could be detected in inactivated sections which were incubated up to 2 hours. (Supported by a grant from the Damon Runyon Memorial Fund for Cancer Research.)

In vivo effects of thiaminase. L. B. WALSH,* D. GREIFF* AND H. PINKERTON. *Dept. of Biology and Pathology, St. Louis Univ., St. Louis, Mo.*

The thiaminase ('Chastek factor' of Green) was prepared by the extraction of dried carp viscera with 10% NaCl. Partial removal of impurities was accomplished by passing the extract, adjusted NpH 4.5, through a 'zeolite' column. The activity of the effluent containing the thiaminase was determined by incubation with known amounts of thiamine (vitamin B_1). After incubation at 37.5° C. for 24 hours, the unreacted thiamine was converted to thiochrome and the amount of the foregoing compound determined by fluorescence under ultraviolet light. The complete enzymatic destruction of the thiamine present was indicated by the absence of fluorescence.

The partial destruction of thiamine was estimated by comparison with the fluorescence of a known amount of B_1. The rate of destruction of thiamine by thiaminase was a function of time; enzymatic activity becomes evident after 8 hours of incubation and reaches an end-point at approximately 24 hours. Daily subcutaneous injections into mice of thiaminase resulted in a temporary hind limb paralysis, disjointed gait and loss of muscle tonus, a syndrome similar to B_1 avitaminosis. One week old chicks treated in the same manner behaved similarly. Liver, kidney and brain tissues of injected chicks were studied for histological changes. The former two tissues showed no microscopic changes. The brain, however, showed a 'dropping out' of Purkinje cells. The relation of enzymatic destruction of thiamine to cellular metabolism will be discussed.

Effect of growth hormone on the development of chick embryo. T. Y. Wang,* K. M. Wang* and H. T. Blumenthal. *Div. of Hormone Research, Labs. of Jewish Hosp. of St. Louis, and Dept. of Pathology, St. Louis Univ., School of Medicine, St. Louis, Mo.*

Growth hormone in saline (pH 8) was injected chorioallantoidally into chick embryos on the 5th, 9th, 13th and 17th incubation day. One group of controls received saline (pH 8) and a second group was sham operated. Experimental eggs were divided into 4 groups, receiving respectively 5, 10, 20 and 40 μg. A high mortality rate was encountered in control and experimental groups injected or operated on the 5th and 9th incubation days; the only survivors were in the groups receiving growth hormone. In chick embryos receiving growth hormone on the 13th incubation day, 10 μg. has been found to be the optimal dosage for maximal hatchability, which was slightly higher than in control groups; increasing hormone beyond this dosage inhibited hatchability, but hastened hatching time. Administration of growth hormone in large doses or on the 17th incubation day resulted in a greater percentage hatchability in controls than in experimental eggs; many of the latter showed small perforations of the egg shell on the day before hatching but the embryos died. Chick embryos receiving growth hormone on the 13th incubation day showed generally higher body weights and blood sugar levels than controls, and the degree of response could be directly correlated with dosage. Saline injected controls showed lower body weights and blood sugar levels than sham operated controls. Females appeared to have a more marked response than males; in females, body weight and blood sugar appeared to have reached a maximum with 40 μg. growth hormone, whereas in males the increases with this dosage were apparently not maximal. This appears to be the first report of a response to growth hormone in aves.

Hepatic injury in mice fed a yeast-protein diet. W. Lane Williams and Richard B. Aronsohn.* *Dept. of Anatomy, Univ. of Minnesota, Minneapolis.*

A high incidence of massive hepatic necrosis has been described frequently in rats fed a (British) yeast-protein diet (Himsworth and Glynn). In the present study 43 hybrid mice (Strong A × Bagg albino) were restricted to a yeast-protein diet when 6–10 weeks of age. Samples of the same diet had produced hepatic necrosis in rats under essentially the same laboratory conditions. Twenty-one mice showed massive necrosis of the hepatic parenchyma after feeding of the diet for 33–272 days. In 2 mice small areas of necrosis were present at 85 and 105 days. In 20 mice fed the diet for 10–283 days necrosis did not occur, but hepatic parenchymal cells showed the following cytoplasmic alterations: *1*) reduction in ribonucleic acid as studied by the ribonuclease-hydrolysis-basophilic staining procedure and by staining with gallocyanin-chromalum at pH 2.5; *2*) liposis (sudanophilia); and *3*) increased alkaline phosphatase activity (modified Gomori procedure). An earlier occurrence and higher incidence of necrosis was observed in mice restricted to the diet when 6 weeks of age.

Protective action of sulfa drugs against injury to liver of mouse by CCl_4. J. Walter Wilson. *Dept. of Biology, Brown Univ., Providence, R. I.*

We and others have reported that the inclusion of some sulfa drugs in the diet protects the livers of mice from damage by CCl_4. The sulfa drugs are not all equally effective. The experiments here reported were conducted to test various hypotheses as to how the drugs produce the effect. Since sulfaguanidine is one of the most effective drugs, it seemed possible that the change in intestinal flora might be responsible. This was ruled out because sulfasuxidine and formo-sulfathiazole, which similarly effect the intestinal flora, have no protective effect. The antithyroid effect of the sulfa drugs was ruled out because the most goitrogenic of all, sulfadiazine, has no protective effect. Furthermore thiourea and related compounds have no protective effect but thyroxine either fed or injected gives pronounced protection. The most complete protection so far found was on a diet containing both sulfaguanidine and desiccated thyroid. The sulfa drugs are in part metabolized by acetylation, some more completely than others. That this accounts for the difference in their ability to protect against CCl_4 injury has been ruled out in three ways: *1*) The addition of PABA as a competitor for the acetylation mecha-

nism did not alter the protective effect of sulfanilamide. *2*) Pantothenic acid deficiency, which is reported to deprive the acetylation mechanism of an essential component, did not alter it. *3*) Elkosin, which is among the least acetylated, has no protective effect. We have, therefore, at present no explanation of the protective effect to offer.

Arterial atheromatous lesions in rat. R. W. Wissler, M. L. Eilert,* M. A. Schroeder* and L. Cohen. *Depts. of Pathology and Medicine, Univ. of Chicago, Chicago, Ill.*

Atheromatous lesions of the coronary arteries and to a lesser degree of the renal arteries and the aorta were observed in about 25% of 63 'middle aged' obese male rats fed synthetic rations for a period of 28–43 weeks. Some of these rations were modeled after the diets of patients who developed coronary thrombosis under 50 years of age. The incidence of lesions in the arteries was almost doubled in 28 additional animals receiving the same rations but also treated with nephrotoxic antiserum followed by daily injections of desoxycorticosterone acetate and the substitution of 1% NaCl solution for their drinking water starting at about 20 weeks. This treatment resulted in a moderate hypertension in the majority of these rats, but it also was followed by a marked increase in serum cholesterol levels and cholesterol: lipid phosphorus ratios. The latter observations seemed to correlate better than the presence or absence of hypertension with the increased incidence of lesions observed in this group. There was no evidence that the atheromatous arterial lesions were related to a variable degree of arteritis seen in some of the animals.

AMERICAN INSTITUTE OF NUTRITION

Sixteenth Annual Meeting

NEW YORK CITY, APRIL 14–18, 1952

(An asterisk following an author's name indicates "by invitation")*

Effects of nonionic surface active agents on the growth of animals. J. B. ALLISON, H. L. ROSENTHAL* AND A. H. MILLS.* *Bureau of Biological Research, Rutgers Univ., New Brunswick, N. J.*

An agar gel diet is described for dogs, mice and hamsters. The surface active agents, sorbitan monostearate (Span 60), polyoxyethylene sorbitan monostearate (Tween 60) and polyoxyethylene monostearate (Myrj 52) added to this diet did not affect the growth or maintenance of these animals unless the concentration was sufficiently high to cause gastrointestinal disturbances and or a reduced food intake. This critical concentration was approximately 15% or more (dry weight) in the diets used. Protein efficiencies (grams gained in body weight per gram nitrogen intake) for the diet containing casein averaged 11.4 for male, 11.0 for female puppies, 8.2 for male, 8.0 for female mice, and 11.6 for male hamsters. The addition of these surface active agents did not alter the efficiencies from control values. The caloric intakes per kilogram of body weight decreased in a regular way from time of weaning to maturity. The data obtained using puppies demonstrated that nitrogen balance per gram of nitrogen intake decreased with age, a decrease which was consistent with the filling of protein stores. Similarly body weight gain per unit of nitrogen balance decreased with age indicating a shift in characteristics of growth as the animal approached maturity. Female mice were grown, bred, and maintained normally through gestation and lactation on diets containing 1 to 15% Myrj 52.

Apparent phosphate requirement for choline oxidase activity. CHARLES ANGEL AND O. NEAL MILLER (introduced by WENDELL H. GRIFFITH). *Dept. of Biochemistry and Nutrition, Univ. of Texas Med. Branch, Galveston.*

It has been observed here and in other laboratories (WILLIAMS, J. N., JR. *et al. J. Biol. Chem.* 192: 73, 1951) that choline oxidase (CO) activity in liver homogenates is not inhibited by addition of betaine. Usually (CO) activity is determined manometrically, assuming that choline causes an additive increase over endogenous oxygen consumption. It appears to us that the end product of an enzymatic reaction should inhibit the rate of reaction and the effect of adding choline may not be additive. Rat liver homogenates are prepared with phosphate, veronal and citrate buffers (all at pH 7.8); centrifuged at 25,000 *g* at 5° C. The residues are resuspended in the appropriate buffer and recentrifuged until each is washed 4 times. Activity of suspensions in citrate and veronal buffers is 18 and 50%, respectively, of the same in phosphate buffer. Addition of phosphate to citrate- and veronal-suspensions stimulates (CO) activity by 25 and 50%, respectively. Evidence that phosphorylation is involved in choline oxidation is obtained by adding dinitrophenol (10^{-3} M) to the veronal-suspension and observing that phosphate continues to stimulate (CO) activity. In an attempt to devise a manometric assay for (CO) which has low endogenous oxygen consumption, cyanide and methylene blue are added. Methylene blue inhibits (CO) due to the high zinc content of commercial preparations of methylene blue. When methylene blue is replaced by phenazine methosulfate, it is found that (CO) activity is some 15 to 50% higher, depending upon the choline concentration, than by the usual methods.

Effect of large oral dosages of aureomycin on the splenic lesions developed by rats depleted of folacin. CONRADO F. ASENJO AND A. POMALES-LEBRÓN.* *Depts. of Biochemistry and Nutrition and of Microbiology, School of Medicine, Univ. of Puerto Rico, San Juan.*

The following experiment was designed to give information on the effect of aureomycin on the splenic lesions reported by one of us (ASENJO, *J. Nutrition* 36: 601, 1948) in folacin depleted rats, especially in the light of our later finding that the necrotic areas of these infarct-like-lesions contain bacteria. Two groups of 21-day-old rats were used. Both received a basal diet free of folacin containing 2% succinylsulfathiazole. One group served as negative controls and the other received in addition aureomycin, 3 mg/day orally. After 31 days on this régime the animals were sacrificed and their spleens removed. Of the 18 aureomycin-treated animals, 6 exhibited the splenic lesion

while of the 16 negative controls, only 1 developed the lesion. In both groups of animals the splenic lesions contained bacteria. The fact that the aureomycin group showed a higher incidence of splenic lesions than the control group should not be interpreted at the present time as meaning that aureomycin enhances the incidence of this condition, because it has been our experience in the past, that negative control animals develop this lesion very irregularly. It can be concluded, however, that, under the conditions of this experiment, aureomycin does not prevent the formation of splenic lesions in folacin depleted rats.

Effect of various dietary proteins on the cholinesterase activity of plasma and brain tissue of rats. CHARLES H. BARROWS* AND BACON F. CHOW. *Dept. of Biochemistry, School of Hygiene and Public Health, Johns Hopkins Univ., Baltimore, Md.*

Our previous communications indicated that oral administration of casein to protein depleted dogs resulted in the regeneration of both albumin and globulin. Feeding of lactalbumin per os stimulated the production of albumin alone unless it was supplemented with a fraction obtained from tryptic digest of casein. These results therefore demonstrated that besides the essential amino acids the dietary protein preparations may contain a factor exerting a 'directive' influence on the synthesis of tissue proteins. The present study was designed to ascertain any effect of dietary proteins on the production of enzymes as exemplified by cholinesterase in certain tissues of rats. Eight groups of weanling rats (male and female) were fed, *ad libitum*, diets containing 20% of the following proteins: meat, egg albumin, lactalbumin, casein, soybean, liver, wheat gluten with or without supplementation with lysine and methionine. They were weighed at regular intervals and sacrificed after 150 days of feeding. Heparinized plasma and homogenized brain specimens were assayed for cholinesterase contents according to the method of Ammon. Our data demonstrate that the cholinesterase contents of plasma of female but not male rats differed markedly, according to the dietary protein offered, but independently of their nutritive values. Furthermore, this difference existed even among certain groups of animals which grew at essentially the same rate and were of the same age and body weight at the time of sacrifice. However, no difference was observed in the cholinesterase activity of brain regardless of sex and dietary proteins.

Extension of the glucostatic scheme of regulation of food intake to 'Houssay' animals, diabetes and other special cases. MARGARET W. BATES* AND JEAN MAYER. *Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.*

In previous publications the authors have demonstrated that in normal animals, relatively short-term variations of blood sugar within physiological limits suffice to significantly affect food intake. A 'glucostatic' scheme of regulation of food intake, involving levels of blood glucose and hypothalamic receptors was thus postulated. A series of experiments was undertaken to bring striking demonstration in special cases and to account for apparent contradictions, as in overeating associated with hyperglycemia. In one such experiment, making use of the fact that prolonged hyperglycemia can readily be induced in Houssay animals, hypophysectomized rats made diabetic by administration of alloxan were used. Fasting blood glucose levels are low in these animals. Intraperitoneal administration of 3 cc. of 20% glucose solution induced a hyperglycemia lasting on the average 10 hours. Two such injections daily induced in a group of rats a fall in food intake of the order of one half during the 12-day period of treatment. Food intakes went back immediately to normal pretreatment amounts when treatment was discontinued. Three daily injections eliminated almost totally any intake of food for a period of 4 days. Similar experiments with diabetic animals, as well as observations of obese, diabetic mice, have led to the concept of glucostatic regulation by 'effective' blood glucose levels.

Contribution of protein, fat and carbohydrates to the caloric intake of obese individuals. RACHEL BEAUDOIN* AND JEAN MAYER. *Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.*

Adequate data on the food habits of overweight subjects are unavailable. The relative proportion of protein, fat and carbohydrate in the diet of obese individuals was carefully studied. The sources of data were: *a*) 1-day food intakes obtained on 125 adult women, obese and normal, as part of the Groton, New York, survey conducted by Cornell Workers in 1948; *b*) 1-day food intakes on 50 obese women determined by the 24-hour recall method routinely used in nutrition clinics; *c*) the diet histories of 30 obese and normal-weight women studied by the method of Burke. The calculations were carried out by means of the Food Composition Table for Short Method of Dietary Analysis of Leichsenring and Donelson Wilson. The diet history method was found superior in disclosing individual intakes and gave more reliable averages. By all 3 methods it appears that on the average the food intake of the obese subjects has a distribution pattern similar to that of the normal lean subjects observed. Specifically, the obese individuals do not consume a larger proportion of carbohydrates in their diets than do normal subjects. Both groups choose from 35 to 40% of their calories as fat rather than the

25% generally regarded as normal for adults on moderate caloric intake.

Biosynthesis of amino acids in lactating dairy cows from $C^{14}O_2$. A. L. BLACK (introduced by MAX KLEIBER). *School of Veterinary Medicine, Univ. of California, Davis, Calif.*

Isotopic studies of CO_2 fixation in dairy cows have demonstrated that 4% of the casein carbon is derived from CO_2 (KLEIBER *et al. J. Biol. Chem.*, in press). An investigation was made to determine the distribution of the CO_2 carbon among the amino acids of the casein molecule. A chromatographic column of Dowex-50 was used to separate the amino acids of the casein hydrolysate by HCl elution. All amino acids contained some CO_2 carbon but the greater recovery was in the nonessential amino acids: alanine 17% (I), aspartic acid 12% (II), glutamic acid 8.5% (III), serine 13%, arginine 5%, proline 2.4%, glycine 2% of the total carbon derived from CO_2. Only 30% to 70% of the carbon-14 was recovered from the carboxyl carbons of (I), (II), and (III) suggesting pathways in addition to the tricarboxylic acid cycle exist for CO_2 fixation in these amino acids. Among the essential amino acids, CO_2 furnished the following percentages of carbon: histidine 0.9% (IV), tyrosine 0.5% (V), valine 0.9% (VI), lysine 0.6%, phenylalanine 1.4%, and leucine 2.3%. 100% of the C-14 was recovered from the carboxyl carbon of (IV), (V) and (VI) suggesting that a reversible decarboxylation results in CO_2 fixation in these molecules. Rates of CO_2 utilization for amino acid synthesis will be discussed.

Studies with chicks receiving suboptimum amounts of vitamins with and without antibiotics. L. G. BLAYLOCK,* L. R. RICHARDSON AND C. M. LYMAN.* *Texas Agricultural Exper. Station, College Station, Tex.*

The effects of suboptimum amounts of vitamins, with and without antibiotics, on the growth of chicks and the free amino acids in the plasma were investigated. A semisynthetic diet was used for pyridoxine, pantothenic acid, riboflavin, choline and niacin; a soybean meal diet for vitamin B_{12}; a synthetic diet containing casein for thiamine and folacin; and a synthetic diet containing egg albumin for biotin. Penicillin and aureomycin had essentially the same effect. The antibiotic increased the growth rate when the diet contained adequate amounts of all vitamins, but very little or no effect when it contained inadequate amounts of pyridoxine, pantothenic acid or niacin. The antibiotic increased the growth rate with inadequate amounts of all other vitamins. The plasma from chicks receiving the synthetic diets contained approximately twice as much valine and 3 times as much methionine as that from chicks receiving the semisynthetic or soybean meal diets. Plasma from chicks receiving inadequate pyridoxine contained more valine, while that from chicks receiving inadequate B_{12} contained less valine and methionine than controls. In 9 tests with 160 chicks receiving inadequate amounts of pyridoxine, the valine content of the plasma averaged 253, as compared to 190 μg/ml. for 9 control groups. In 4 tests with 80 chicks receiving inadequate amounts of vitamin B_{12}, the valine content of the plasma averaged 120, as compared to 144 μg/ml. for the controls. The corresponding values for methionine were 27 and 35 μg/ml. The values for other amino acids will be reported.

Diet as a factor in the production of degenerative changes in tissues of rat. ELIZABETH C. CALLISON, MURRAY FISHER* AND ELSA ORENT-KEILES. *Bureau of Human Nutrition and Home Economics, Agricultural Research Administration, U. S. Dept. of Agriculture, Beltsville, Md.*

Studies were made to determine the physiological response of the laboratory rat to diets in which egg replaced varying amounts of a nutritionally adequate basal diet. Substitution of egg up to 25% of the diet promoted development of degenerative changes of greater severity and at an earlier age than in rats fed egg alone, egg plus calcium, or stock diet. The most frequent lesions were encountered in the kidneys; there was a high incidence also of myocardial scarring, calcification of heart muscle, aorta, and several of the major arteries; spontaneous tumors appeared in a considerable number of animals. There was evidence of elevated blood pressure in the animals examined. The acceleration of physiological impairment observed on the high egg diets is probably the result of multiple long-continued dietary stresses produced by an imbalance among essential nutrients, or of changes with increasing maturity in the ability of the animal to metabolize certain food constituents, rather than an effect of egg per se. These results may indicate that nutritional excesses are fully as damaging to the adult animal as nutritional deficiencies. Similar studies in which other foods are substituted for eggs in the experimental diets are in progress.

Tracer studies of the biological precursors of glycine. P. C. CHAO,* C. C. DELWICHE* AND DAVID M. GREENBERG. *Div. of Biochemistry, Univ. of California School of Medicine and Div. of Plant Biochemistry, Univ. of California, Berkeley.*

When glycine-1-C^{14}-N^{15} or glycine-2-C^{14}-N^{15}, together with sodium benzoate, was injected intraperitoneally into rats, the hippuric acid excreted in the urine had the same ratio of C^{14} to N^{15} as that of the labeled glycine injected. This intact utilization of glycine in the animal implied that a normal precursor or precursors of glycine in the rat must supply glycine as a whole. Dietary glycine, serine and threonine are the only natural

amino acids which can fulfill this requirement. Allothreonine, like glycolic acid, is a good precursor when available. Lactate, on the other hand, is a very poor one, as is also fructose, thus excluding a direct conversion from carbohydrate. When 0.1 mEq. of glycine-1-C^{14}/kg. of rat was injected intraperitoneally, upon entering the liver about 11% was immediately dilute adbout 10-fold by the free glycine present. One-seventh of this amount was converted into liver protein in 24 hours. For giving daily injections of glycine-1-C^{14} it was determined that the maximum amount of incorporation into liver protein glycine was about $\frac{1}{800}$ the total glycine content of the liver protein, indicating that the injected glycine was further diluted 80 times in 24 hours. This gave a half life of 4 hours for the free glycine in liver. The half life of liver protein was about 5 days and the liver protein degraded in 24 hours produced about an 11-fold dilution of the free glycine.

Influence of copper on iron absorption. M. S. Chase,* C. J. Gubler, G. E. Cartwright and M. M. Wintrobe. *Dept. of Medicine, Univ. of Utah College of Medicine, Salt Lake City.*

Weanling albino rats were fed a diet of cow's milk supplemented with 0.5 mg. of iron (ferrous chloride) and amounts of copper (cupric chloride) ranging from none to 1000 μg/day/animal. When the group receiving no copper became anemic, radioactively tagged iron was substituted for nonradioactive iron. Total body copper and radio-iron were then determined. The amount of isotopic iron absorbed was 3.6, 10.6, 16.5, 19.8 and 11.1%, respectively, in rats given 0, 50, 250, 500 and 1000 μg. of copper/day. Thus, up to amounts of 500 μg. of copper daily, the quantity of iron absorbed was greater as the amount of copper fed was increased. In another experiment 50 animals were fed 0.5 mg. of iron and 50 μg. of copper/rat/day for 3 weeks. A single dose of radio-iron was then administered while the amount of added copper was changed to range from zero to 1000 μg/day. Under these conditions the amount of radio-iron absorbed was the same in all of the animals. In a third experiment a constant amount of iron was fed but different groups of rats received 0, 50, 250, 500 and 1000 μg. of copper daily for 5 weeks. The administration of copper was then stopped and a single dose of radio-iron given. The greatest amount of iron was absorbed when 250 μg. of copper were given. Thus, under the conditions of these experiments, the amount of copper in the tissues influences the amount of iron absorbed.

Liver glycogen of the rat after food restriction and rehabilitation on a high protein diet unsupplemented or supplemented by vitamin B_{12}. Ruth Clayton* and Esther Da Costa. *U. S. Army Med. Nutrition Lab., Chicago, Ill.*

The average liver glycogen of 288 male rats fed either a control, high (60%) protein, low calorie high (10%) salt, or modified carrot (2% protein) diet for 35–70 days was 2.5, 2.0, 1.2, 2.0 and 4.3 gm/100 gm. of tissue, respectively. Restricted animals were rehabilitated for $\frac{3}{4}$, 2, 3, 7, 35 or 70 days. After $\frac{3}{4}$ day of rehabilitation following 35 days on the low calorie diet, the liver glycogen rose to 140 to 440% above the low calorie level. After 7 days of rehabilitation following 70 days on the low calorie diet, liver glycogen varied from 140% above to 50% below the low calorie level. After 35 days on the carrot diet, the liver glycogen with $\frac{3}{4}$–7 days of rehabilitation, was reduced to 5–22% of the depletion level. After 35 or 70 days of rehabilitation following 35 or 70 days on a low calorie diet, liver glycogen was essentially at the control level. After protein depletion and subsequent rehabilitation during a similar period, liver glycogen was significantly above the control level. In early rehabilitation after caloric restriction, increased glucoenogenesis may result from increased adrenocortical activity, since liver glycogen gains and increased adrenal weight paralleled each other. Increased glycogen deposition occurred later in rehabilitation following protein depletion, probably because time was needed to build up protein reserves for hormone synthesis.

Importance of relative humidity in vitamin deficiencies. R. A. Collins,* M. Schreiber,* H. Niño H.* and C. A. Elvehjem. *Dept. of Biochemistry, Univ. of Wisconsin, Madison.*

Weanling rats fed a complete synthetic diet and diets deficient in certain vitamins have been reared at the following relative humidities: 10, 50 and 90%. The growth rate and the vitamin deficiency symptoms of the animals were observed. Rats fed the complete diet and the pyridoxine deficient diet grew more rapidly at high humidity that at low. A scaly dermatosis was observed on the paws of rats fed the complete diet and the diets deficient in vitamin A, riboflavin, pyridoxine, and pantothenic acid at a relative humidity of 10%. A spontaneous recovery from the dermatosis was observed in the animals receiving the complete diet. No dermatosis was observed on the paws of the rats at 50% relative humidity. Blood-caked whiskers were observed on the vitamin deficient animals at high humidity. Pantothenic acid deficiency produced the most severe excretion of this red pigment. In general rats were least active at low humidity and showed the greatest activity at high humidity.

Bioautographic analysis of extracts of blood from various species. J. R. Couch and V. M. Doctor.* *Texas A. and M. College System, College Station*

Aqueous extracts of autolyzed blood samples from various species were chromatographed on paper strips and analyzed by bioautography. *Lactobacillus leichmannii* 4797 was used in the seed organism. A normal butanol-water system was used in making the chromatograms. Liver extract and vitamin B_{12} were used as reference standards. Bioautographs of extracts from autolyzed blood samples of the turtle, chicken and turkey blood were almost identical. There was a stationary spot at the top which corresponded to vitamin B_{12} and three spots below. The latter was also almost identical with a bioautograph of liver extract, except that the liver extract contained B_{12b} in addition to B_{12}. If the above mentioned blood samples were autoclaved at pH 7.0 and bioautographed, the stationary spot was the only spot observed on the bioautograph. The latter indicates that desoxyribonucleases in the blood accounted for the liberation of the desoxyribosides in blood from species with nucleated erythrocytes. Aqueous extracts from autolyzed blood samples from the turtle, chicken and turkey, when autoclaved at pH 12.0, gave a bioautograph showing the three desoxyribosides only. Bioautographs of extracts from autolyzed blood samples from the cow, pig and sheep showed a B_{12} spot but not the desoxyribosides.

Utilization of nitrogen in the absence of adequate calories. WARREN M. COX, JR., RUDOLPH C. ELLINGSON* AND ARTHUR J. MUELLER.* *Mead Johnson Research Labs., Evansville, Ind.*

As a consequence of the fact that nitrogen utilization is improved by an increased intake of calories, it has been held that the caloric requirement has priority over the nitrogen requirement and that a deficit of calories leads to the catabolism of exogenous protein for energy, rather than for tissue synthesis. The present experiment was designed to determine to what extent protein could be used for growth when the caloric intake was inadequate. Fifty-two groups of 6 rats each were fed dextrose and isocaloric quantities of mixtures of dextrose and protein hydrolysate (Amigen). Adequate vitamin and minerals were supplied. Weight changes, water intake and the final body composition of normal, protein depleted, calorically restricted and scalded rats fed 10, 20, 30 or 50 calories daily for 5 weeks were observed. At all levels of caloric intake the protein depleted animals receiving nitrogen gained more weight, or lost less weight, than the groups receiving dextrose alone, and total body protein paralleled the weight changes. For example, when fed 10 calories ($\frac{1}{3}$ caloric need) as dextrose they lost 16 gm., but with the same calories as dextrose and Amigen they lost only 4 gm. Similar differences, of greater magnitude, were observed at each calorie level and with each series of animals. Further studies of the ratio of protein to carbohydrate to produce best growth at each level of caloric intake are in progress.

Estimation of amino acid requirements of swine from tissue analyses. L. V. CURTIN,* JEAN ABRAHAM,* H. H. WILLIAMS, J. K. LOOSLI AND L. A. MAYNARD. *Depts. of Biochemistry and Nutrition and Animal Husbandry, Cornell Univ., Ithaca, N. Y.*

A study has been completed in which values for the amino acid requirements for growth of swine were estimated by determining the proportions of the essential amino acids and tyrosine in the bodies of pigs. Two weanling pigs, 2 pigs weighing approximately 33 kg. and 2 market pigs were analyzed for the 10 essential amino acids and tyrosine. The average amino acid analyses of the body tissues of the pigs expressed in gm/16 gm. of nitrogen and the relative proportions among the amino acids using lysine as 100 are arginine 6.94, 83; histidine 2.71, 32; isoleucine 3.95, 47; leucine 7.06, 84; lysine 8.39, 100; methionine 1.79, 21; phenylalanine 3.73, 44; tyrosine 2.56, 31; threonine 3.70, 44; tryptophan 0.73, 8.8; and valine 5.83, 70, respectively. The estimated requirements of the amino acids for growth calculated from the known requirement of the pig for lysine (5.5% of protein), previously determined, and the average proportions existing between the amino acids and lysine in the bodies of the pigs are arginine 4.6, histidine 1.8, isoleucine 2.6, leucine 4.6, methionine 1.2, phenylalanine plus tyrosine 4.1, threonine 2.4, tryptophan 0.5, and valine 3.9% of the protein. The values are in close agreement with the values that previously have been estimated by growth studies for histidine, isoleucine, methionine and tryptophan.

Serum alkaline phosphatase of male rats during food restriction and subsequent protein rehabilitation. ESTHER DA COSTA AND RUTH CLAYTON.* *U. S. Army Med. Nutrition Lab., Chicago, Ill.*

Serum alkaline phosphatase activity, measured in 288 male rats after 35 or 70 days on a control, high (60%) protein, low calorie, high (10%) salt, or modified carrot (2% protein) diet was 65.6, 82.9, 56.6, 114.6 and 25.3 units/100 ml. of serum, respectively. After either 35 or 70 days of restriction, the rats were rehabilitated for $\frac{3}{4}$, 2, 3, 7, 35 or 70 days on either a high protein diet fed *ad libitum*, or supplemented by B_{12} (7.5 μg/rat) or 11 gm/rat/day. Serum alkaline phosphatase activity rose gradually or fell during early rehabilitation. After 7 days of rehabilitation following 35 days on the low calorie diet, serum alkaline phosphatase rose from 68 to 85% above the low calorie level and after 7 days of rehabilitation following

35 days on the carrot diet, serum alkaline phosphatase rose from 68–143%. After 7 days rehabilitation following 35 days on the high salt diet, alkaline phosphatase was below the high salt level, but after 70 days of rehabilitation, from 30–41% above. The slowest recovery of serum alkaline phosphatase occurred in the groups given B_{12}. After 35 days of rehabilitation, it reached the control level in most rats. After 70 days, it mostly exceeded the high protein control level. A parallel rise in body weight and muscle and heart protein suggests that serum alkaline phosphatase activity serves as a measure of nutritional status.

The apparent vitamin B_{12} content of eggs as affected by extraction in the presence of cyanide. C. A. DENTON,* W. L. KELLOGG* AND H. R. BIRD. *Bureau of Animal Industry, U. S. Dept. of Agriculture, Beltsville, Md.*

Vitamin B_{12} was estimated by the microbiological procedure set up by the U. S. Pharmacopoeia. The vitamin was extracted by autoclaving the yolks (whites contain little or no B_{12}) with sodium acetate buffer at pH 4.5 for 30 minutes at 15 pounds, with and without sodium cyanide. When cyanide was not used, yolks from hens on an adequate diet gave an average value of .12 μg. of B_{12}/yolk (range .07–.15). With cyanide present, the value was .48 μg. (range .34–.56). When 1 μg. of B_{12} was added to each sample the average B_{12} value obtained was .84 μg/yolk, or a recovery of about 75%. However, when cyanide was present during the extraction, an average value of 1.4 μg. was obtained, which was a recovery of 95%. In yolks from hens depleted of B_{12} the average value without cyanide was .02 μg/yolk (range .01–.027). With cyanide, the yolks assayed .11 μg. of B_{12} (range .07–.18). When 1 μg. of B_{12} was added/yolk, without cyanide present, the average assay value was .81 μg. which represented a recovery of 79%. With cyanide a value of 1.1 μg. was obtained which was about 99% recovery. Similar effects with cyanide have been obtained with tissues and feces as well as with eggs. It appears that the usual methods for the extraction of B_{12} do not make available to the test organism all the added or naturally occurring B_{12} of eggs, tissues and feces.

An elevated xanthine oxidase in vitamin E deficient rabbits. JAMES S. DINNING (introduced by PAUL L. DAY). *Dept. of Biochemistry, School of Medicine, Univ. of Arkansas, Little Rock.*

Rabbits were given a purified diet in order to produce a deficiency of vitamin E. Controls were given this diet with oral supplements of alpha tocopherol acetate, other controls were given a diet of commercial rabbit chow. Homogenates of livers from the control animals exhibited very low xanthine oxidase activities; in contrast, homogenates of livers from vitamin E deficient rabbits exhibited very high xanthine oxidase activities. These results are a further indication of an influence of vitamin E on the metabolism of nucleic acids (DINNING, SEAGER AND DAY, *Federation Proc.* 10: 380, 1951; YOUNG AND DINNING, *J. Biol. Chem.* 193: 743).

Occurrence of a conjugated form of leuconostoc citrovorum factor (CF). V. M. DOCTOR* AND J. R. COUCH. *Texas A. and M. College System, College Station.*

Aqueous solutions of yeast extract (Difco), Liver Fraction L (Wilson), Liver concentrate 1-20 Paste (Wilson), and Liver Injection 2 U.S.P. Crude (Wilson) were chromatographed on paper strips with wet symmetrical collidine as the developing agent. The ½ inch paper strips after drying were cut length wise into two ¼ inch strips. One of these was bioautographed on nutrient agar containing purified chick pancreas enzyme. The other was bioautographed on nutrient agar which did not contain the enzyme. Nutrient agar was seeded with *L. citrovorum* 8081 in all instances. Paper strips were incubated on the surface of the agar for 16 hours at 37° C. The data obtained show that yeast extract and liver fraction L contain a conjugated form of CF which appears to be identical in both cases. The bioautographs of these samples show a stationary component on the nutrient agar plate with added chick pancreas enzyme but is absent at the equivalent positions on the nutrient agar plate not containing the enzyme. The stationary components (conjugated CF) moved at the same rate when chromatographed with a mixture of ethanol, butanol, ammonia and water as the developing agent and bioautographed as described above.

Effects of diet, partial hepatectomy and growth promoting factors on the composition of the rat liver. MARY E. DUMM* AND ELAINE P. RALLI. *Dept. of Medicine, College of Medicine, New York Univ.-Bellevue Med. Center, New York City.*

Adult rats were maintained on a 9% protein, 38% fat diet for 42 days and were then partially hepatectomized. The liver removed was analyzed for lipids, nitrogen and solids. After operation, the rats received the same diet without any supplement or with injections of liver extract or vitamin B_{12}. Other rats were transferred after operation to 16 or 26% protein (9% fat) diets. The animals were sacrificed 6–15 days after operation and the analyses repeated. In the rats continued on the 9% protein diet with no supplement liver lipids increased and nitrogen decreased from operation to sacrifice. Injections of liver extract or increasing the protein in the diet had some lipotropic effect and also increased liver nitrogen. Vitamin B_{12} had a pronounced and consistent lipotropic effect associated with an increase in

liver nitrogen. Microscopically the livers of rats on the low protein diet with no supplement showed increased lipid deposition at sacrifice. Rats given liver extract or the higher protein diets showed decreased liver fat and evidences of cellular regeneration. Rats receiving vitamin B_{12} showed striking evidences of cellular regeneration.

Pharmacological value of flavonoids in capillary fragility. WALTER H. EDDY AND BORIS SOKOLOFF.* *Southern Bio-Research Lab., Florida Southern College, Lakeland.*

Using capillaroscopy, the protective action of flavonoids against radiation injury to the capillaries of the nail bed was demonstrated. Two hundred r delivered in a single dose induces considerable alterations of the capillary wall and formation of petechiae; 600 mg. of citrus flavonoid compound given for 10 days prior to the exposure gives an almost complete protection to the capillary wall of the nail bed against 200 r. No petechiae are formed and the capillary structure remains intact. The analysis of 300 case histories reported by 22 radiotherapists indicates that citrus flavonoid compound reduces radiation erythema up to 60–90% and increases considerably the tolerance to deep radiation. Citrus flavonoid compound reduces hemorrhages induced by bacterial polysaccharide and increases the span of life of animals from 7 hours 44 minutes, average, to 46 hours. The analysis of 35 case histories reported by 4 tuberculosis sanatoria indicates that citrus flavonoid compound promptly arrests in almost all cases persistent hemoptysis of tuberculous patients and that bleeding from the lung is reduced to a minimum under flavonoid therapy. The therapeutic value of citrus flavonoids in increased capillary fragility induced by bacterial toxins or radiation is revealed by these observations.

Methionine in the maintenance of the structure of hepatic tissue. CECILE HOOVER EDWARDS* AND PEARL SWANSON. *Nutrition Lab., Iowa Agricultural Exper. Station, Ames.*

Previous reports from this laboratory have suggested that methionine occupies a key position in the metabolic processes of adult male rats depleted of body reserves of protein and force fed nitrogen-low diets of varying fat and caloric values. The present study suggests that the effect of dietary methionine may have been mediated through provision of a critical moiety for the elaboration of vital hepatic proteins. For instance, at adequate caloric intakes in the absence of methionine, hepatic cells were swollen, and the cytoplasm was missing or clumped together in parts of the cell. The cells maintained their characteristic staining qualities, however. Fat and glycogen were present in greater than normal quantities. The tissue following the feeding of inadequate calories showed faintly staining areas around the central vein and periphery of the hepatic lobule. Cellular outlines were indistinct: nuclei stained faintly, if at all. Alterations in total hepatic nitrogen and the ratios, respectively, of riboflavin and niacin to total nitrogen suggested the presence of abnormal protein. The improvement in hepatic histology induced by supplementation of the diet with methionine was striking. The cells were similar to those of the tissue of the normal rat; cellular membranes were clear and distinct. Cytoplasm was fairly evenly distributed. The ratios of riboflavin and niacin to total nitrogen were normal.

Appearance of radioactivity in milk of rats after oral and parenteral administration of vitamin B_{12} containing cobalt^{60}. R. C. ELLINGSON,* A. J. MUELLER,* WARREN M. COX, JR. AND B. F. CHOW. *Mead Johnson Research Labs., Evansville, Ind., and Dept. of Biochemistry, School of Hygiene and Public Health, Johns Hopkins Univ., Baltimore, Md.*

With the availability of vitamin B_{12} containing cobalt^{60} and the development of a satisfactory method of milking small animals it became possible to determine the appearance of radioactivity in milk of rats after oral and parenteral administration of vitamin B_{12}*. Two groups of lactating rats were given 300 or 500 mμ. of vitamin B_{12}* orally and a 3rd group 300 mμ. subcutaneously. The rats were milked at various intervals (2–16 hours) thereafter and the radioactivity of the milk measured. Each of the 54 rats was milked only once; the volume was 0.5–4.0 cc/rat. No radioactivity was found in milk of 28 of the animals receiving vitamin B_{12}* orally and only traces in milk of the other 10. Large amounts of radioactivity were found in milk of 15 rats receiving the vitamin subcutaneously; a trace was found in the milk of one. There was no correlation between the quantity of radioactivity in the milk and the interval between administration of the vitamin and milking of the rats. The mothers were returned to their young and subsequently 1 rat from each litter was killed. The radioactivity of the kidneys, liver and carcass was determined. None could be detected in these tissues. Assuming radioactivity of the milk to be a measure of vitamin B_{12}*, at least 3.5 times as much B_{12}* is secreted/cc. of milk by rats receiving the vitamin subcutaneously than by those receiving it orally.

Prevention of nutritional edema with vitamin B_{12} and folic acid. R. W. ENGEL AND H. D. ALEXANDER.* *Dept. of Animal Husbandry and Nutrition, Alabama Polytechnic Inst., Auburn.*

A choline-low diet containing 7% of protein produced a high incidence of generalized nutri-

tional edema and severe anemia in weanling rats in an average period of 90 days. The edema and anemia were prevented by supplementing the diet with 0.2% of choline chloride (ENGLE AND ALEXANDER, *Federation Proc.* 10:382, 1951). The edema was completely prevented and hemoglobin levels of 11 gm/100 ml. of blood were maintained for 300 days when the choline-low diet was supplemented with 30 μg. of vitamin B_{12} and 2 mg. of folic acid/kg of diet. Rats receiving this supplement made average body weight gains of 120 gm. during the experiment, contrasted with average body weight gains of only 20 gm. in rats protected from edema with a supplement of choline. A supplement of folic acid alone did not prevent the edema or the anemia although it appeared to prolong survival. Rats receiving this supplement failed to grow and developed edema in 120 days, at which time the blood hemoglobin levels had decreased to between 2.2 and 6.1 gm/100 ml. of blood.

Nutritional status of Iowa school children. ERCEL EPPRIGHT,* CHARLOTTE RODERUCK AND MILICENT HATHAWAY. *Iowa State College and Bureau of Human Nutrition and Home Economics, Ames.*

Nearly 700 children representing all elementary schools in Iowa and junior and senior high schools in cities under 50,000 were subjects for this investigation, which is part of a larger study including Ohio and Kansas school children. Seven-day dietary records, blood levels of hemoglobin and serum levels of vitamin C, carotenoids and alkaline phosphatase were obtained. Less than 5% of these Iowa children had hemoglobin below 11.5 gm.%, over 40% had ascorbic acid below 0.6 mg.%, and nearly 30% had carotenoids under 80 μg.%. In general, the picture was satisfactory with respect to hemoglobin, but not ascorbic acid and carotenoid concentrations. Children 12 years and older were in less satisfactory status with respect to vitamin C and carotenoids than younger children. The peak alkaline phosphatase values for girls occurred in the 9-10-11-year-old group, the peak for boys in the 12-13-14-year-old group. In contrast with boys, girls 15 years of age and older had attained adult alkaline phosphatase levels. Preliminary analyses of the diets showed liberal intakes of meats, cereals, and potatoes but less than recommended amounts of milk and of vitamin C- and carotene-rich fruits and vegetables. Few children had dietary supplementation with vitamin D. Food habits of older children, particularly girls, were often inferior to those of younger. Blood constituents and dietary pattern of Ohio and Kansas children will be presented later. (Contribution of North Central Regional project NC-5.)

Protein nutrition in highly inbred strains of mice. PAUL F. FENTON, MARIAN T. DOWLING* AND JOHN B. LYON, JR.* *Brown Univ., Providence, R. I.*

Mice of 4 different highly inbred strains (C57, A, C3H and I) have been tested for their response to diets containing 5, 10, 30, 50, 70 or 90% casein. C3H and A strain mice grew equally well on the 10 and 30% protein diets. On the other hand C57 and I strain mice grew significantly better at the 30 than at the 10% level. Protein efficiency ratios have been determined both at the 5 and at the 10% protein level. At both levels of protein intake the efficiency ratios were higher for the C3H mice than for the C57 or I. Mice of the C3H strain consumed markedly more food than did the other 2 strains. Therefore mice of this strain, as well as A strain mice, were restricted in their food intake to that of the C57 mice. Protein efficiency ratios were again determined under these conditions. The occasionally large individual variations in efficiency of food utilization for growth were found to be largely due to differences in body weight at the time of weaning. Mice of smaller than normal weaning weights exhibited inordinately great efficiency of food utilization for growth. Dietary protein levels greater than 30% inhibited growth in proportion to the casein content of the diet. This inhibition was markedly less for the C57 strain than for the other 3. A variety of dietary supplements have been tested for their ability to overcome the growth inhibition of the high protein diets with only slight success. Measurements of oxygen consumption have been carried out in an effort to shed some light on the causes of the differences in food intake.

Influence of dietary fat on nitrogen metabolism in the protein-depleted rat. HAZEL METZ FOX,* S. P. YANG* AND PEARL SWANSON. *Nutrition Lab., Iowa Agricultural Exper. Station, Ames.*

Tissue catabolism in rats fed a low calorie diet adequate except for its protein content is augmented when fat is removed from the ration. In previous tests demonstrating this phenomenon, the ration contained 10% each of butter fat and lard. Food was offered *ad libitum*. Nitrogen balance was used as the index of measurement. Whether or not this body-sparing action is common to all fats was studied in the present investigation. Protein-free diets containing either butter fat, lard, vegetable oil, hydrogenated fat, margarine, or various combinations of these fats were formulated. The fat content of the various rations was maintained at 20%. When the nitrogen metabolism of rats fed these diets reached a steady state, the quantity of ration offered was reduced so that it provided only ¼ the number of calories

normally ingested. One hundred fifty-six rats were used in the test. Under the conditions of this experiment, a marked difference in the protective action of fats was apparent. Average nitrogen balances in a 5-day interval ranged from −343 mg. to −657 mg. depending upon the source of fat in the diet.

Function of vitamin B_T (carnitine). G. FRAENKEL. *Dept. of Entomology, Univ. of Illinois, Urbana.*

Work on the effect and isolation of vitamin B_T has been previously reported and its identity with carnitine has been established (*Federation Proc.* 10: 170, 183, 1951; *Arch. Biochem. Biophys.* In press). Carnitine has hitherto only been known to occur in muscle, but with the greatly more sensitive biological testing method with *Tenebrio*, it was found almost universally in biological material. Requirement of B_T in the diet has so far been established only for 3 insect species, but other insects have been shown to synthesize it. Many animal tissues contain it in relatively large amounts, but it is in all probability synthesized in vertebrates. Egg contains little of B_T, but appreciable quantities are synthesized in the chick embryo. It is contained in many materials of vegetable and microbial origin, but has not been demonstrated in *E. coli* and *Tetrahymena*. Muscle is by far the richest known source, with meat extracts containing 1–2%. In *Tenebrio* the function of B_T seems to be intimately connected with processes occurring during a molt; most deficient animals die immediately after a molt. The pathology of the B_T-deficient *Tenebrio* will be described. Figures will be presented on the excretion of carnitine in the human urine and the possible function of carnitine to replace choline will be discussed. It is concluded that carnitine must have 2 distinct functions, 1 requiring large amounts, and which is reflected by the large amounts in muscle, and 1 requiring the minutest amounts which is reflected by its almost universal occurrence.

Dietary fat and carbohydrate in reproduction and lactation. C. E. FRENCH, R. H. INGRAM,* L. K. KNOEBEL* AND R. W. SWIFT. *Dept. of Animal Nutrition, Pennsylvania State College, State College.*

Extending the investigation of the role of fat in the diet (*J. Am. Oil Chem. Soc.* 26: 171, 1949) to include life cycle studies, the reproduction and lactation performance of the albino rat was measured. The control diet consisted of commercial rat diet and experimental diets were composed of 80% commercial rat diet plus 20% of sucrose or 20% of corn oil. The experimental diets were supplemented with liver, yeast, choline, tocopherols and thiamin to supply adequate amounts of known and unknown factors. With an average of 80 matings in 3 generations for each diet a significant decrease in reproduction performance of rats receiving the fat diet was observed:

	NO. YOUNG/LITTER		BIRTH WEIGHT (GM.)	
	Alive	Surviving	Male	Female
Control	10.0	9.8	6.5	6.0
Carbohydrate	10.2	10.0	6.4	6.0
Fat	9.3*	9.0*	6.1*	5.7*

Lactation performance, measured on an average number of 350 young in 3 generations for each diet (all litters reduced to 6 young on the 3rd day) revealed no decrease due to the fat diet. However, a significant improvement in lactation resulted from the carbohydrate diet:

	WEIGHT GAIN (GM.) 3–14 DAYS AGE		WEANING WEIGHT (GM.) 21 DAYS AGE	
	Male	Female	Male	Female
Control	20.3	19.8	44.5	42.4
Carbohydrate	22.0*	21.1*	48.8*	46.7*
Fat	20.3	19.8	44.6	43.5

Rat growth assay for vitamin B_{12}. Correspondence with collaborative microbiological assay results on U.S.P. liver extracts. DOUGLAS V. FROST, HOWARD H. FRICKE* AND HENRY C. SPRUTH.* *Abbott Labs., North Chicago, Ill.*

A rat growth assay for vitamin B_{12} using a highly purified diet with casein has been further studied and improved. Rats used in this study were from the laboratory colony. These animals, originally Wistar, have been inbred over a period of 20 years. Animals purchased from 1 outside source responded satisfactorily while those purchased from 2 other suppliers did not. It is therefore clear that maternal diet is important in the preparation of weanling rats for this assay. Two liver extract samples, used in 1950 U.S.P. collaborative study of the microbiological vitamin B_{12} assay, were assayed in rats at levels corresponding to average B_{12} values of 23 participating laboratories. The growth responses for the 2 liver extracts at 3 levels in the diet were in close agreement with the response to similar graded levels of crystalline vitamin B_{12}. The method was used to test the specificity of the microbiological method as a measure of the completeness of destruction of vitamin B_{12} in liver extract by treatment with sodium hydroxide. Results, which were in agreement between rat and microbiological assay, indicated that destruction of vitamin B_{12} was not entirely complete even under conditions of drastic treatment. The antithyrotoxic factor of Ershoff does not appear to complicate this rat growth assay for vitamin B_{12}. Nicotinamide added

to the diet depressed the growth of control and supplemented animals, but did not necessarily improve the sensitivity of the method.

The time factor in utilization of dietary amino acids. E. GEIGER, I. EL RAWI* AND LESLIE E. GEIGER.* *Univ. of Southern California Med. School and Van Camp Labs., Terminal Island.*

One of the authors reviewed recently (*Federation Proc.* 10: 670, 1951) the evidences which indicate that protein is utilized optimally for growth when the supply of amino acids parallels closely the growth requirements of the tissues. This point was further investigated by the following experiments: Male Long-Evans rats (weight, 70–80 gm.) were divided into groups of 8 aniamls each. *Group 1* received from 11:00 p.m. until 5:00 a.m. a basal diet (containing carbohydrate, fat, vitamins, minerals) to which 15% of a casein hydrolysate has 767—2-6-52 FP 5541 p. 37 Take 2-11-8 cx gal.11 been added (spray dried acid hydrolysate plus 1% tryptophan plus 0.5% cystine). *The body weight of these animals increased 3–5 gm. daily. Groups 2* to *4* received a protein free basal diet from 11:00 p.m. to 5:00 a.m. Besides this, the animals of these groups received by gavage the protein hydrolysate through stomach tube as follows: *Group 2* at 12:00 noon, 1½ gm. casein hydrolysate in 4 ml. water; *no growth. Group 3* at 8:00 a.m., at noon and at 4:00 p.m. each, 0.5 gm. casein hydrolysate in 1.3 ml. water; *no growth. Group 4* at 9:20 a.m., 10:40 a.m., 12:00 noon, 1:20 p.m., 2:40 p.m. and 4:00 p.m. each, 0.25 gm. protein hydrolysate in 1 ml. water. *Rats of this group gained daily 2–3 gm.* These experiments show that the 1½ gm. protein hydrolysate which supports growth when admixed to the diet does not promote growth when fed apart from the basal diet either in 1 dose or when fed divided into 3 equal doses at 4-hour intervals. Given in 6 equal doses at 80-minute intervals, growth promotion occurs. These experiments support the contention that short-lasting, superoptimal supply of amino acids cannot be utilized efficiently for growth. The fate of the amino acids given in 1 or 3 doses which did not support growth is presently investigated. (Supported by the Williams-Waterman Fund of the Research Corporation.)

Effect of amino acid imbalance on the course of Lansing poliomyelitis in mice. S. N. GERSHOFF, A. F. RASMUSSEN, JR. AND P. F. CLARK (introduced by C. A. ELVEHJEM). *Depts. of Biochemistry and Med. Microbiology, Univ. of Wisconsin, Madison.*

An effort has been made to increase the resistance of mice to poliomyelitis by feeding rations designed to produce amino acid imbalances. The sparing effect of amino acid deficiencies and of 6-methyl tryptophan have been reported previously from this laboratory. In this work the course of Lansing poliomyelitis in mice on rations containing 5-methyl tryptophan, 6-methyl tryptophan, α-ethyl tryptamine, α-amino-β(3-indazole) propionic acid, methionine sulfoximine, low tryptophan and excessive tryptophan, methionine, choline, leucine, lysine, histidine and phenylalanine has been studied. In most cases, the mice were placed on the experimental diets for a week before being inoculated intracerebrally with Lansing strain poliomyelitis virus. The most significant results were obtained with low tryptophan, added 6-methyl tryptophan and excessive amounts of methionine. The use of these dietary regimes resulted in longer incubation and survival times and, in some cases, increased the number of survivors. The effect produced by combining these 2 rations was greater than that produced by either alone.

Incomplete recovery of the rat after early retardation in growth. MARIANNE GOETTSCH. *Dept. of Biochemistry and Nutrition, School of Medicine, Univ. of Puerto Rico, San Juan.*

If a diet, consisting of polished rice and red kidney beans, is given to the dam during the period of lactation, and then to the young during the period of early growth, the young rats grow very slowly and do not attain sexual maturity. Upon realimentation with an adequate diet at the age of 60 days, the rate of growth, reproduction and lactation compare favorably with those of littermate controls on the adequate diet from the time of birth. The rats are not able to attain the adult body weight of the species.

Influence of the protein level and of manganese on bone weight, total calcium content and percentage calcium. RAYMON M. GREENBERG,* MARIE GREENE BARRY* AND JEAN MAYER. *Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.*

In a series of experiments, groups of 8 weanling rats were put on 2 series of diets, 1 containing 600 mg.% amounts of calcium, 1 containing 200 mg.%. In each series, 5 levels of dietary protein were used, 6, 10, 14, 20 and 40%. Another group of 8 weanlings were sacrificed at weaning time for calcium analyses of both femurs. After 20 and 40 days, groups of 6 rats corresponding to each diet were sacrificed and analyses performed on both femurs for each animal. Results show that increased protein levels led, in all cases, to heavier bones, to larger amounts of calcium deposited, and, more remarkable, to increased percentage weight of calcium. Differences between high and low calcium groups increased when the protein level increased. The differences also increased with time, being several times greater after 40 than after 20 days. These differences could not

be accounted for by effects on growth, optimal weight being reached at the 14% protein level; they could be accounted for only in part by differences in absorption. In a second series of experiments, the effect of partial manganese deficiency was studied with a similar experimental set up. Decreases in calcification were obtained with decreases in manganese concentration. Differences increased with time, on both levels of calcium and at all levels of protein. Implication of differences in phosphatase activity is indicated.

Iodine in nutrition. ISIDOR GREENWALD. *Dept. of Chemistry, New York Univ. College of Medicine, New York City.*

A recent review (*Handbook of Nutrition*, American Medical Association, 1951) repeats the false claim that goiter in Yunnan, China was due to the cessation of the previous supply of salt from the coastal provinces due to the Japanese occupation. The claim is also made "that the thyroids from the goiterogenous regions are the largest and have . . . the lowest iodine content." The authority given does not confirm this statement. The thyroids from patients in Columbus, Ohio, not known to have been treated with iodine, contained an average of 11 mg. Reports by others from various nongoiterogenous regions, including Japan, give averages of 8.1–11 mg. The excretion of iodine in the urine is said to be higher in nongoiterogenous regions than in those in which goiter is prevalent. In order to obtain the values that were compared, the high excretion reported by Lunde (Second International Goiter Conference) for a goiter region in Norway was not included and the high figures reported from Danzig are included as from a nongoiter region. It was precisely the high incidence of goiter in iodine-rich Danzig that led Liek to reject the 'iodine-lack' hypothesis in the very paper from which these data were taken.

Role of copper in erythropoiesis. C. J. GUBLER, M. E. LAHEY,* M. S. CHASE,* G. E. CARTWRIGHT AND M. M. WINTROBE. *Depts. of Medicine and Pediatrics, Univ. of Utah College of Medicine, Salt Lake City.*

Swine made deficient in copper, like iron-deficient swine, develop a hypochromic microcytic anemia, normoblastic hyperplasia of the bone marrow, hypoferremia, and an increase in the iron-binding capacity of the plasma. These changes occur in copper-deficient swine in spite of the oral administration of large amounts of iron from the beginning of the experiment. Following the administration of copper there is an increase in the plasma iron from hypoferremic to normal or hyperferremic levels within 24 hours. This is then followed by reticulocytosis and disappearance of the anemia and other abnormalities. By the use of radioactive iron it has been possible to demonstrate that in copper-deficient swine, the absorption of iron from the gastrointestinal tract is decreased. Corroborating this finding is the observation that the iron content of the tissues of the copper-deficient swine is decreased. Impairment of iron absorption does not, however, adequately explain the development of anemia in copper-deficient animals since the anemia does not respond to the parenteral administration of large doses of iron. Furthermore, only about 25% of radioiron administered intravenously is utilized for hemoglobin formation. These results suggest that in copper-deficient swine the ability to properly metabolize iron is impaired and as a consequence of this an anemia, which is morphologically and chemically indistinguishable from iron-deficiency anemia, results.

Serum ascorbic acid, vitamin A, carotene, vitamin E, riboflavin and alkaline phosphatase values in Central American school children. MIGUEL GUZMÁN* AND NEVIN S. SCRIMSHAW. *Inst. of Nutrition of Central America and Panama, Guatemala, C. A.*

Blood sera from Central American school children in the 7–12 age range were studied preparatory to the introduction of supplementary feeding programs. In Guatemala 275 school children had average values as follows: ascorbic acid, 1.24 mg.%; vitamin A, 27.2 γ%; carotene, 121 γ%; vitamin E, 0.61 mg.%; riboflavin, 1.78 γ%; alkaline phosphatase, 5.14 mM/l/hr. with para-nitrophenyl phosphate substrate. In El Salvador the average values on 200 school children were: ascorbic acid, 1.77 mg.%; vitamin A, 21.7 γ%; carotene, 68 γ%; vitamin E, 0.69 mg.%; riboflavin, 1.57 γ%; alkaline phosphatase, 4.94 mM/l/hr. In Honduras 90 school children averaged: ascorbic acid, 2.64 mg.%; vitamin A, 28.6 γ%; carotene, 57 γ%; vitamin E, 0.67 mg.%; riboflavin, 1.73 γ%; alkaline phosphatase, 5.43 mM/l/hr. In Costa Rica the values on 140 school children averaged: ascorbic acid, 1.47 mg.%, vitamin A, 28.8 γ%; carotene, 77 γ%; vitamin E, 0.74 mg.%; riboflavin, 1.46 γ%; alkaline phosphatase, 4.66 mM/l/hr. The vitamin A and carotene results appeared related to clinical and dietary findings. The high ascorbic acid levels as well as rural and urban differences are discussed. No cases with elevated (greater than 8 mM/l/hr.) alkaline phosphatase were observed. Vitamin E and riboflavin values do not differ from those for school children in the United States. These values are apparently not influenced by 20-γ tablets of vitamin B_{12} or 50-mg. capsules of aureomycin administered 6 days/week over periods up to 15 months.

Unsaturated fatty acid levels in human serum. ARILD E. HANSEN AND HILDA F. WIESE.* *Dept.*

of Pediatrics, Univ. of Texas Med. Branch, Galveston.

Failure to determine a possible relationship between essential fatty acids and human nutrition has in large part resulted from lack of a suitable method for measuring diene, triene and tetraene fatty acids in small quantities of blood and tissues. The development of a semimicro technique for determination of unsaturated fatty acids in serum employing 7- to 15-mg. samples (3 ml. of serum) instead of 100-mg. samples as in macro methods seemed to offer the opportunity long sought for. Hence, the fasting serum level of 2, 3 and 4 double bond fatty acids was determined in 75 human subjects. Whereas most of the human subjects were hospitalized patients, they all fell in 1 of 2 groups. In 1 group, the individuals were either normal adult subjects or hospitalized children who had had adequate diets and appeared to be in a good state of nutrition clinically. The 2nd group was composed of children markedly underweight. The most characteristic finding was the high level of diene fatty acid in the serum of well nourished children and adults. These values were above 27% of the total fatty acids. In the children who were underweight and considered to be markedly malnourished from the clinical viewpoint, the serum levels of diene fatty acid were in the range of 6–14% of the total fatty acids. About ⅓ of all the human subjects studied had a serum level of diene fatty acid between 14 and 23% of the total fatty acids. All the patients in this group were suffering from eczema or from disorders characterized by poor fat absorption—the celiac syndrome. Values for the triene and tetraene fatty acids tended to vary in the same direction as for the diene fatty acids but to a lesser degree. None of the patients studied had elevated temperatures at the time the serum lipids were studied. (Support for this work was received by a contract sponsored by the Bureau of Human Nutrition and Home Economics, U. S. Department of Agriculture and authorized by the Research and Marketing Act.)

Disappearance of radioactivity from various organs of normal adult rats following subcutaneous injection of tagged vitamin B_{12}. ROBERT A. HARTE,* BACON F. CHOW AND LOIS BARROWS.* *Research Div., Sharp and Dohme, Inc., West Point, Pa. and Dept. of Biochemistry, School of Hygiene and Public Health, Johns Hopkins Univ., Baltimore, Md.*

Following the earlier demonstration that subcutaneously injected radioactive (Co^{60}) vitamin B_{12} led to retention of radioactivity in the tissues of various organs (kidney, liver, muscle, etc.) even after 2 weeks, estimation of the rate of disappearance of such concentrations of radioactivity becomes of interest. Thirty healthy adult rats, both males and females, from the stock colony were injected, subcutaneously, with a single dose of 2 μg. of tagged vitamin B_{12} (specific activity, 60 μc/mg.). The animals were kept in individual metabolism cages. Urine specimens were collected after 1, 7 and 14 days and assayed for vitamin B_{12} by the microorganism adsorption technic of Davis and Chow. Groups of 5 animals (2 males and 3 females) were sacrificed at intervals and various organs and tissues carefully dissected out. Following acid digestion, radioactivity of the cobalt salts was determined. The findings indicate that after the first day more radioactivity is localized in the kidneys than elsewhere, but this amount tended to decrease sharply with time. Lesser absolute amounts but much higher concentrations of activity were found in the pancreas. The amounts of radioactivity in liver, pancreas, muscle and spleen remained essentially constant or even increased with time. Vitamin B_{12} in liver is apparently associated with the protein moiety since the total amount of radioactivity remains constant but the concentration rises when the liver glycogen is mobilized as a result of caloric deprivation.

Growth retarding effect of unheated soybeans. C. H. HILL* AND R. BORCHERS. *Dept. of Agricultural Chemistry, Univ. of Nebraska, Lincoln.*

While the growth retardation due to feeding unheated soybeans has long been recognized, the mechanism of the inhibition is obscure. Although an impaired amino acid absorption from unheated soybeans has been suggested, studies in this laboratory indicate that unheated soybeans retard the growth rate of New Hampshire chicks when adequate amounts of all essential amino acids are present in addition to those supplied by the soybeans. In order to test for a possible toxic factor in unheated soybeans increasing levels of unheated and heated soybeans were fed to chicks. The soybeans replaced casein to provide a constant level of protein in this experiment. There was no difference in the retardation of chick growth when the level of unheated soybeans was increased from 30–70% of the diet. Twenty per cent of unheated soybeans caused a greater growth retardation than did the higher levels. It would appear, therefore, that the improvement of soybeans by heating is not due to a simple destruction of some toxic factor.

Effect of yeast on biological activity of vitamin A and β-carotene. E. L. HOVE. *Dept. of Animal Husbandry and Nutrition, Alabama Polytechnic Inst., Auburn.*

Koehn (*Arch. Biochem.* 17: 337, 1948) found β-carotene to be biologically equal to vitamin A on a weight basis. This differs from the official and accepted relative activities according to which

vitamin A is twice as active as β-carotene in the rat-growth bioassay. In the work of Koehn, the most obvious deviation from the U.S.P. bioassay technique was the omission of yeast from the diet. Therefore, the influence of this diet ingredient on the biological response to pure vitamin A and β-carotene has been studied. On a semi-synthetic vitamin A deficient diet, without yeast, repeated bioassays showed β-carotene to have 70–90% of the activity of vitamin A over a response range of 0.25–1.0 μg/day, with 1 mg. daily of DL-α-tocopherol. With 8% of dried yeast in the diet bioassays run concurrently showed β-carotene to have only 40–50% of the activity of vitamin A. Yeast in the diet allowed greater growth responses to vitamin A over the entire range of the assay and even at high levels. On the other hand, yeast diminished the growth response to β-carotene. The factor in yeast responsible for this phenomenon occurs in the lipid fraction but is apparently not related to the fat soluble antioxidant of unknown nature occurring abundantly in yeast oil.

Further in vivo and in vitro studies on Maillard reaction. M. IACOBELLIS, H. LEES AND L. J. SCHROEDER (introduced by ARTHUR H. SMITH). *Dept. of Physiological Chemistry, Wayne Univ., Detroit, Mich.*

Evidence was shown in previous studies in this laboratory that autoclaving whole raw milk at 10 or 15 lb. pressure for 15 or 30 minutes has no detrimental effect on the nutritive efficiency of the contained protein. These results stimulated further work to find the explanation for the observed beneficial effect of heat on the milk protein. The fat content and the aqueous medium in which proteins and carbohydrates are dispersed were the factors which fell under consideration. Commercial 'Starlac' which is obtained from high quality fresh whole milk by removing 97.5% of H_2O and 99% of fat was the experimental food. This product was autoclaved at 15 lb. for 30 minutes as such and in the presence of various amounts of water. The utilization of the protein was determined by nitrogen balance studies on 3 adult dogs. 'Starlac,' which was autoclaved dry, kept the dogs in negative nitrogen balance as contrasted to the postive nitrogen balance observed with 'Starlac' autoclaved in the presence of water. It appears that although the browning reaction may lower the nutritive value of food proteins by decreasing their digestibility, under the experimental conditions of concentration and degree of heating, the Maillard reaction is not as much in evidence when the reactants were in an aqueous medium. This *in vivo* finding was followed by *in vitro* enzymatic digestibility studies on the same 'Starlac' heated and reconstituted under the same conditions as the *in vivo* studies. The data obtained essentially confirmed the observations obtained in the nitrogen balance studies.

Factors involved in utilization of homocystine by chicks. THOMAS H. JUKES. *Lederle Labs. Div., American Cyanamid Co., Pearl River, N. Y.*

Vitamin B_{12}-depleted chicks were fed diets deficient in methionine, choline and vitamin B_{12}. The response to homocystine was markedly increased by vitamin B_{12} under various dietary conditions. However, in the absence of vitamin B_{12} homocystine often depressed growth. The presence or absence of vitamin B_{12} or folic acid had little or no effect on the response to methionine. Choline appeared to be highly effective as a 'methylating' agent for homocystine in the presence of vitamin B_{12} for chicks on a diet markedly deficient in methionine. The response to vitamin B_{12} was greater in the presence of folic acid than in its absence and was greater in the presence of homocystine than in its absence when methionine was omitted. The response to folic acid was usually diminished by adding choline. The addition of homocystine and vitamin B_{12}, or homocystine and choline to the basal diet gave poor growth, but excellent growth was obtained when homocystine, vitamin B_{12} and choline were all three added. With vitamin B_{12} added, betaine was much less effective than choline in improving the response to homocystine unless dimethylaminoethanol was added, in which case betaine was almost as effective as choline. Sodium formate or additional glycine had little effect on the utilization of homocystine for growth in the presence of vitamin B_{12}. No response to methionine was obtained in chicks deficient in both pyridoxine and methionine. The requirement for pantothenate was not significantly affected by vitamin B_{12}-deficiency in chicks on a diet adequate in methionine and choline.

Improvement of milled rice proteins by buttermilk and whey proteins. MARINUS C. KIK. *Univ. of Arkansas, Fayetteville.*

The proteins of milled rice rations (percentage composition milled rice, 87; salt mixture, 4; cellu flour, 2; vegetable shortening, 4; sucrose-vitamin B complex mixture, 1; corn oil-vitamin A, D, E mixture 1; sucrose-biotin mixture, 1; and cerelose) were supplemented by those of buttermilk and whey. Six male and 6 female rats were used in experiments of 70 days' duration. The supplements were fed at a 1, 3, and 5% level replacing an equivalent amount of protein in the milled rice rations which had a protein content of 5.43%; milled rice, 6.24%; buttermilk, 32.3%; whey, 12.25% protein. The average *difference* in gain per 1000 grams of food intake at the 1, 3, and 5% level was for buttermilk −7.5, +3.5, and +10.0 grams

respectively. For whey −6.8, +3.9, and −2.6 grams. These differences are considered not to be significant.

Amino acid requirement of young women. I. Threonine. R. M. LEVERTON, M. R. GRAM,* E. BRODOVSKY,* A. MITCHELL* AND M. CHALOUPKA.* *Human Nutrition Lab., Nebraska Agricultural Exper. Station, Lincoln.*

The subjects were 8 college women between the ages of 19 and 24 years. A dietary foreperiod of ordinary foods which supplied 6 gm. nitrogen daily was followed by a purified diet of amino acids, mineral salts, vitamins, liver extract, sugar, cornstarch and butterfat. Six subjects were on this purified diet for 46 days and 2 subjects for 39 days. Except for threonine the amino acid composition of the diet was equivalent to 15 gm. of egg protein plus aminoacetic acid to bring the total daily nitrogen intake to 5 and then 6 gm. The L-threonine intake was reduced stepwise from 735 mg., to 367 mg., to 184 mg., to 73 mg., to 0 mg., and then 147 mg. of the DL form was fed. The time on each level varied from 5–16 days depending on the nitrogen excretion which was determined daily throughout. Variation in urinary nitrogen was often great enough to obliterate differences in nitrogen balance on different levels of L-threonine intake. However, 184 mg. L-threonine maintained nitrogen equilibrium in all subjects fed that level. In 3 subjects 73 mg. were sufficient. Mean daily negative nitrogen balances on 0 mg. L-threonine ranged from −0.53 gm. to −1.06 gm. Six subjects fed 147 mg. DL-threonine did not consistently have balances equal to their balances on 73 mg. L-threonine.

Prolonged percutaneous administration of sex hormones on growing male rats. A. E. LIGHT AND J. A. TORNABEN (introduced by G. R. COWGILL). *The Wellcome Research Labs., Tuckahoe, N.Y.*

The importance attached to the balance between male and female sex hormones in aging processes led to this study of the effects of methyl testosterone and estradiol on growing male rats. The cutaneous route of administration was chosen because of a possibly more constant release of the hormone into the system and because of interest in maximal dermal effects. Three groups of 5 animals each (36–42 gm.) were placed in oblong cages and given free access to ground Purina fox chow and water. The rats were shaved on the back weekly and the animals from each group inuncted daily for 201 days with the respective hormone or placebo ointment. Growth rates and food and water consumption were recorded along with urine and fecal excretion data for intermittent periods. Differences in the above values due to temperature changes were also noted. Estradiol increased food and water consumption while decreasing weight gain and male organ development. Higher temperatures (29°–30°) lowered food intakes, especially for the estrogen treated animals. Terminal histological studies gave evidence of enlarged pituitaries and adrenals in the estrogen group along with other changes. The kidneys of the rats treated with either hormone were enlarged, especially those following methyl testosterone inunction. It may be postulated that in males an excess of female hormone leads to great energy expenditure, increased appetite for food and water and minimal fat deposition.

Nicotinic acid metabolite excreted by rats given radioactive nicotinic acid or nicotinamide. PEI-HSING LIN* AND B. CONNOR JOHNSON. *Div. of Animal Nutrition, Univ. of Illinois, Urbana.*

The radioactive nicotinic acid metabolite previously identified (*Federation Proc.* 10: 203, 1951) as N′-methyl-nicotinamide on paper strip chromatograms of the rat urines has been found to be a combined spot of N′-methyl-nicotinamide and trigonelline. These compounds have been separated by two dimensional paper chromatography using a butanol-acetone-water solvent system followed by a morpholine-butanol-water system. The two dimensional chromatograms give characteristic pattern of flow for the N′-methyl-nicotinamide and for the trigonelline. Following C^{14}-nicotinic acid or amide injection both compounds were excreted containing C^{14} and were detected by radioautographs. Also the N′-methyl-nicotinamide gives a bright violet fluorescence with the morpholine of the second solvent while the trigonelline does not. The trigonelline gives a growth response with *Torula cremoris* on a bioautograph while N′-methyl-nicotinamide does not. Among the unidentified compounds previously mentioned (*loc. cit.*) one accounting for 0.48 and 2.4% of the radioactivity of the urine of rats following C^{14}-nicotinic acid and C^{14}-nicotinamide injection respectively has been spectrophotometrically identified as N′-methyl-2-pyridone-5-carboxylamide (cf. LEIFER *et al. J. Biol. Chem.* 190: 595, 1951). Another unidentified spot accounting for 7.7 and 1.0% of the C^{14} in the urine following nicotinic acid and nicotinamide administration respectively has been found to be a conjungated compound in which the ring nitrogen is so bound that the compound does not react with CNBr. On acid hydrolysis two radioactive compounds are formed, one of which gives a positive ninhydrin reaction and the other responds to *Torula cremoris*.

Synthesis of cystine from inorganic sulfate by the hen. L. J. MACHLIN,* C. A. DENTON,* P. B. PEARSON AND H. R. BIRD. *Bureau of Animal*

Industry, U. S. Dept. of Agriculture, Beltsville, Md.

A single dose of carrier-free S^{35} labeled sulfate was injected intramuscularly into 2 hens. S^{35} activity in the eggs laid was determined for 10 days. During that time, approximately 1.2% of the original dose was found in the albumens and 1.0% in the yolks. Three hens were given daily injections of S^{35} labeled sulfate. Radioactive sulfate accounted for about 53% of the activity in the albumen and 80% of the activity of the yolk of eggs laid by these hens. The albumen and yolk of several eggs were dialyzed individually for 5 days against distilled water. The nondialyzable portion was hydrolyzed. Radioactive cuprous cysteine mercaptides were isolated from the hydrolysates. Control studies demonstrated that there was no contamination with sulfate or methionine. A radiochromatogram of an albumen hydrolysate confirmed the presence of radioactive cystine and indicated that no radioactive methionine was present.

Control of cholesterol metabolism in rabbits. GEORGE V. MANN, HILDA WHITE* AND F. J. STARE. *Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.*

There is no satisfactory explanation for the frequently observed differences of response of individual rabbits to cholesterol feeding. When the large dose of 1 gm. of cholesterol per day is fed, the individual differences will be manifest as differences in the severity of the serum cholesterol defect and perhaps in differences of degree of involvement of the aorta and visceral organs. It has been shown that smaller daily doses of cholesterol will accentuate the individuality of rabbits in their response to cholesterol feeding. With a daily dose of 200 mg. of cholesterol per day and with other conditions constant (i.e. caloric intake, weight change, total fat intake) animals of a commercial strain may be classified according to their serum cholesterol responses. A few animals do not respond with hypercholesterolemia while at the other extreme are a few animals which respond within a few weeks with high and increasing serum levels. Furthermore, with experimental conditions constant there are often large and unexplained fluctuations of the serum cholesterol levels. Experiments in which lipid balances were carried out have revealed that these fluctuations of the serum cholesterol coincide with changes of lipid balance, the serum levels varying inversely with the fecal loss. Contrary to hypothesis, caloric restriction regularly leads to pronounced elevations of the serum cholesterol levels and reduction of the fecal loss of lipids. The mechanism of these changes is being studied.

Physical measurements of school children. ABBY MARLATT,* MARY BROWN PATTON,* ERCEL EPPRIGHT* AND MILICENT HATHAWAY. *Kansas State College, Manhattan, Ohio State University, Columbus, Iowa State College Ames, and Bureau of Human Nutrition and Home Economics, Washington, D. C.*

Physical measurements were obtained on approximately 1,830 9-, 10- and 11-year-olds in Kansas, Iowa, and Ohio, by trained workers following common techniques and using similar equipment. Standing height was measured to the nearest tenth of centimeter with the child backed up against a support. Weight with indoor clothing was obtained to the nearest ounce on platform scales. Amount of clothing worn was noted and correction made for it. Chest and bi-iliac diameters in centimeters were made with calibrated sliding calipers. Calf girth was measured in centimeters with a steel tape. Means, ranges, and standard deviations of these measurements will be presented in relation to age and sex. Percentage deviations from the Baldwin-Wood, Lucas-Pryor, and Stuart-Meredith standards will be shown. The data are being plotted on the *Wetzel Grid* for classification into 4 groups determined by channel and auxodrome location and for comparison with the frequency distribution reported by Wetzel. These data are of interest in relation to accumulating evidence that children of the present generation may differ in height and weight from those of several decades ago.

Distribution of tocopherols in human tissues. KARL E. MASON, MEI YU DJU* AND LLOYD J. FILER JR.* *Dept. of Anatomy, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

In 20 human fetuses (2–6 months) gestation age, analyzed in toto, the average level of tocopherols (α, γ, δ) was 0.31 mg/100 gm. fresh tissue (range, 0.17–0.55). In 10 other fetuses (3–6 months), values were 0.57 for skeletal muscle, 0.57 for liver and 0.60 for other visceral organs. The average tocopherol content of 19 placentae (2 months to term) was 0.71 mg/100 gm. There was no tendency for tocopherols to increase or decrease in fetal tissue or placentae. In 17 premature infants the average tocopherol values were: lung 0.51, skeletal muscle 0.63, heart 0.85, kidney 0.87, liver 1.14 and adipose tissue 2.14 mg/100 gm. Corresponding values for 6 full-term infants were appreciably lower. In one full-term stillbirth, analyzed in toto, tocopherol concentration was 0.56 mg/100 gm. and total tocopherols 20.15 mg. In 16 infants (3 days–3 years), the average tocopherol content of muscle, liver and adipose tissue was 0.51, 0.83 and 2.41 mg/100 gm., respectively. In 5 individuals 13–17 years of age the corresponding

values were 1.24, 1.30 and 9.25, respectively. Other data indicate that during early and middle adult life tocopherols increase slightly in liver and markedly in adipose tissue. They apparently decrease in later decades of life. In 10 individuals 61–93 years of age (av. 79.6 years), the average tocopherol levels were: skeletal muscle 1.58, heart 1.0, liver 0.92 and adipose tissue 9.92 mg/100 gm. (Supported by a grant from the National Vitamin Foundation, Inc.)

Primary hereditary lesion in obese-diabetic mice. JEAN MAYER, MARGARET W. BATES,* RUTH RUSSELL* AND MARGARET M. DICKIE.* *Dept. of Nutrition, Harvard School of Public Health, Boston, Mass. and Jackson Memorial Lab., Bar Harbor, Maine.*

Some of the metabolic characteristics of a strain of mice showing a Mendelian recessive occurrence of obesity have been described. The obese animals reach a weight up to 2 to 3 times that of their non-obese siblings. They present hyperglycemia, glucosuria and an extremely high degree of insulin resistance. Their blood sugar level goes down quickly when the animals are fasted. They voluntarily choose a high proportion of fat in their diet. Pathological studies reveal cutaneous lesion, centripetal deposition of fat, absence of liver glycogen and enlargement of islets of Langerhans. Studies of respiratory exchanges show that the total basal energy exchange is not raised by obesity; on the average, basal metabolic levels of the obese mice are 40 % below that of the normal mice. Blood cholesterol levels are high. Metabolic exchanges are abnormally sensitive to thyroxine. Food consumption and weight are reduced by thyroxine in the absence of restriction of intakes. Prolonged thyroxine administration also causes an elimination of hyperglycemia and greatly increases insulin sensitivity. Responses of non-obese and obese animals to administration of thiouracil, propylthiouracil, adrenotrophic, gonadotrophic and somatotrophic hormones, prolactin, sex hormones and diethylstilbestrol, goldthioglucose, alloxan, rhothane, to experimental surgery, and to various dietary treatments suggest at the present time that the primary lesion might be a hypersecretion of diabetogenic (adrenotrophic) hormone accompanied by a hyposecretion of other pituitary trophic hormones.

Liver storage of vitamin A in tumor-bearing mice. PATRICIA T. MCCARTHY* AND LEOPOLD R. CERECEDO. *Dept. of Biochemistry Fordham Univ., Bronx, N. Y.*

In view of the fact that many carcinogenic hydrocarbons hasten the depletion of liver stores of vitamin A in the rat, the effect of the potent hepatic carcinogen, 2-Acetylaminofluorene, on liver stores of vitamin A was investigated in the mouse. The prolonged administration of this compound did not interfere with the function of the liver in storing and releasing vitamin A. Nor did the rapid increase in liver protein, the development of widespread tumor formation, and marked increases in nucleic acid levels of various organs which resulted, have any effect upon the hosts' stores of vitamin A. This observation was made throughout the various stages of tumor induction and growth, as well as at widely varying levels of vitamin A intake. The effect of the status of the host with respect to vitamin A upon the development of transplanted tumors was also investigated. This was accomplished by determining the nucleic acid content of the tumors and other tissues of mice bearing transplants of Sarcoma 180 and Lymphosarcoma 6C3HED and maintained on vitamin A free and vitamin A sufficient diets from birth. Changes in the nucleic acid levels were unaffected by the presence or absence of vitamin A from the diet. In spite of the rapid growth exhibited by tumor transplants, such growth had no effect upon the rate of depletion of hepatic stores of vitamin A, nor did a state of vitamin A deficiency lessen the survival time of tumor-bearing mice. (Supported by a grant from the National Cancer Institute.)

Effect of large amounts of ascorbic acid on iron salt-induced folic acid deficiency. ERNEST G. MCDANIEL* AND FLOYD S. DAFT. *National Inst. of Arthritis and Metabolic Diseases, Natl. Insts. of Health, Bethesda, Md.*

It was reported from this laboratory (*Federation Proc.* 10: 387, 1951) that high iron feeding (approximately 3% ferric citrate) in purified diets caused granulocytopenia and reduced growth in rats. Folic acid was effective in preventing the granulocytopenia but not the reduced growth. It was also reported, that 2% of dietary ascorbic acid prevented the development of certain B-vitamin deficiencies in rats. A complete report was given of results with pantothenic acid deficient diets (*Federation Proc.* 10: 380, 1951). We wish to report at this time the effect of ascorbic acid on rats receiving high iron diets. Dietary ascorbic acid at a level of 2% prevented or delayed the development of the granulocytopenia on diets containing 3% or 1% ferric citrate, and in addition improved the growth rate on the 1% ferric citrate diet. The data at hand suggest that parenteral ascorbic acid may prevent or delay the development of granulocytopenia in rats fed the 1% ferric citrate diet. In therapeutic tests dietary ascorbic acid proved to have some value in rats which had developed granulocytopenia on high iron diets.

Unidentified growth factors for chicks. J. MCGINNIS, L. R. BERG AND H. SAXENA (introduced

by A. C. WIESE). *Dept. of Poultry Science, State College of Washington, Pullman.*

Two experiments were conducted with chicks to determine *1*) the effect of sucrose, glucose, corn and wheat in a purified type of diet containing isolated soybean protein on growth response to supplements of fish meal and dried whey and *2*) the response of chicks to graded levels of corn and fish meal and combinations of these ingredients. The results obtained showed that a much greater response to both fish meal and whey was obtained with the diets containing sucrose or glucose than with the diets containing corn or wheat. The responses obtained with fish meal and whey to the two diets containing either corn or wheat were of doubtful significance, whereas, highly significant responses were obtained when sucrose or glucose was used to supply the carbohydrate. In the second experiment, it was found that graded levels of corn and of fish meal gave increasingly greater growth response. The growth response obtained with 15% fish meal was greater than that obtained with 75% of corn. However, the maximum response obtained with combinations of fish meal and corn was only slightly greater than the response to the 15% level of fish meal alone. The possibility that the growth responses were due to recognized vitamins appears to have been ruled out since the basal diets contained levels of the known vitamins higher than the required amounts. These results suggest that the growth response given in these experiments by fish meal and by corn was due to the same factor present in both supplements but in different concentrations.

Comparative urinary excretion of ascorbic, dehydroascorbic and diketogulonic acids in the rat under normal and low temperature conditions. MARY MILLS MONIER AND ROSLYN J. WEISS (introduced by JOSEPH H. ROE). *Dept. of Biochemistry, George Washington Univ. School of Medicine, Washington, D. C.*

The 24-hour excretion of ascorbic acid (AsA), dehydroascorbic acid (DHA) and diketogulonic acid (DKA) was measured in normal adult albino rats under control conditions. An average of less than 20% of the urinary 'ascorbic acid' is in the oxidized forms, DHA and DKA. Normal adult rats were placed in an environmental temperature of 0° C ± 0.5°, and the 24-hour excretion values for AsA, DHA and DKA were measured. Preliminary results indicate a trend toward increased production of the oxidized forms when the animal is in the cold. The effects of cold environment upon the partition of the ascorbic acid and its oxidation products in the urine are discussed.

Modification of choline deficiency by simultaneous pantothenic acid deficiency. AGNES FAY MORGAN AND EUNICE M. LEWIS.* *Dept. of Home Economics, Univ. of California, Berkeley.*

The effect of choline deficiency upon rats fed normal and pantothenic acid-deficient diets was observed from the 14th to the 56th day of life. In the pantothenic acid-deficient and doubly deficient animals the liver fat remained normal or low and adrenal cholesterol much lower than in the normal and choline-deficient groups even when pair-fed. When vitamin B_{12} was given the liver fats were normal in all cases but adrenal cholesterol remained markedly depressed in the pantothenic acid-deficient rats. Neither the addition of 1 % cholesterol to the diet nor the daily injection of adrenocortical extract increased the adrenal cholesterol or the liver fat of animals deprived of pantothenic acid. Blood and serum lipid phosphorus levels were slightly raised in the latter groups. Daily injection of 2 mg. ACTH for 5 days before autopsy produced increases in liver fat of normal animals but none in pantothenic acid-deficient groups. The adrenal cholesterol of ACTH treated animals was somewhat increased but in all the pantothenic acid-deficient rats these levels were less than half that of the normal animals. The thymus weights were greatly reduced in all the pantothenic acid-deficient groups whether ACTH treated or not. Thus, lipid metabolism of pantothenic acid-deficient rats was severely deranged as shown by failure of fat deposition in the liver, loss of adrenal cholesterol, and rise in serum lipid phosphorus. An increase in lipid utilization may occur in this deficiency state.

A comparison of physical-chemical methods with the biological assay of vitamin A. T. K. MURRAY* AND J. A. CAMPBELL. *Food and Drug Div., Dept. of Natl. Health and Welfare, Ottawa, Canada.*

The Morton-Stubbs correction procedure for the assay of vitamin A has been widely accepted. It has been generally agreed that this method yields a conservative estimate of biological potency, but to date little information has been published on this point. In the work to be reported, various vitamin A products of pharmaceutical grade, including cod and halibut liver oils and concentrates, have been assayed spectrophotometrically and colorimetrically by the procedures recommended by the U.S.P. XIV. The results from these assays have been compared against biological potencies as determined by the vaginal smear method of Pugsley *et al.* (*J. Nutrition* 28: 365, 1944) and the U.S.P. XIV growth assay. In general, the corrected results were found to agree more closely with the biological potencies than did the uncorrected or colorimetric results. A cod liver oil has been subjected to progressive oxidation and samples have been removed at intervals and analyzed spectrophotometrically, colorimetrically and biologically. It was found

that until oxidation had progressed to the point where the absorption maximum was shifted from 325 mμ, the correction procedure gave a more accurate estimate of biological activity. In two samples in which extensive oxidation had taken place, the potencies when assessed by either the spectrophotometric or the colorimetric procedures fell outside the confidence limits (P = 0.05) of the bioassay. The possibility of omitting the saponification of vitamin A-containing oils, and of applying a correction directly to the absorption readings on solutions of the whole oil, will be discussed.

Nitrogen balance in adult rat as affected by diets low in L-,DL-, and D-phenylalanine. E. S. NASSET AND ANN M. SILICIANO.* *Dept. of Physiology, Univ. of Rochester School of Medicine and Dentistry, Rochester, N. Y.*

Adult rats were fed a synthetic diet by stomach tube. Amino acids supplied the dietary nitrogen in a mixture which simulated whole egg in its content of essential amino acids. Glutamic acid was substituted for the non essential amino acids. Phenylalanine was reduced in the mixture until it became the limiting factor in the utilization of ingested nitrogen. Nitrogen intake was sufficient nearly to maintain nitrogen equilibrium in one set of experiments and approximately half of this amount in another set. From the results of these experiments the following requirements of phenylalanine for the maintenance of nitrogen equilibrium are computed: 17.0 mg. L, 17.5 mg. DL, and 19.2 mg. D-phenylalanine/day/kg.$^{\frac{3}{4}}$

Relation of anterior pituitary gonadotrophins to pyridoxine deficiency in the rat. MARJORIE M. NELSON, WILLIAM R. LYONS* AND HERBERT M. EVANS.* *Inst. of Experimental Biology and Div. of Anatomy, Univ. of California, Berkeley.*

Previous studies have shown that pregnancy can be maintained in B_6-deficient rats by administration of both estrone and progesterone but not by either hormone separately. To determine whether the production or excretion of pituitary gonadotrophins was involved in these hormonal inadequacies, the pituitary hormones used to maintain pregnancy in the hypophysectomized rat have now been tested. In the uninjected B_6-deficient rat with 21 days of deficiency prior to breeding, 94% of the animals resorbed their fetuses by mid-pregnancy. Daily injections of estrone and progesterone to groups similarly deficient resulted in the maintenance of living young in 80% of the animals. In contrast, injection of the pituitary gonadotrophins, FSH, ICSH (i.e. LH), and MH (mammotrophin) resulted in living young for only one-quarter (27%) of the group. The combination of a single ovarian hormone with the appropriate gonadotrophin(s) was next tried. The use of estrone together with MH gave essentially the same results with 22% having living young whereas the use of FSH and ICSH with progesterone was not as beneficial, only 10% showing living young. The results indicate not only inadequate pituitary production or secretion of the gonadotrophins but also inadequate ovarian response in the B_6-deficient rat to gonadotrophins.

Enzymatic conversion of folic acid to citrovorum factor. CHARLES A. NICHOL (introduced by ROBERT W. HEINLE). *Dept. of Pharmacology, Western Reserve Univ. School of Medicine, Cleveland, Ohio.*

Studies of the factors influencing the metabolic alteration of folic acid (FA) by rat liver slices showed that the presence of ascorbic acid increased the yield of the product, citrovorum factor (CF), and aminopterin inhibited the reaction (C. A. NICHOL and A. D. WELCH, *Proc. Soc. Exper. Biol. & Med.* 74, 52 and 403, 1950). Conditions suitable for the activity of this system in homogenates have been found. When incubated with phosphate buffer under nitrogen, homogenates of rat liver (optimal pH 6.5) and chick liver (optimal pH 6.2) were capable of forming as much CF as liver slices. Under aerobic conditions the reaction proceeded very slowly in homogenates. The presence of ascorbic acid caused a consistent increase in the yield of CF derived from FA. Since this system may permit detailed study of the donors of the hydrogen atoms and the formyl group involved in the conversion of FA to CF, concentration of the enzymes concerned has been undertaken. Active extracts have been obtained from acetone powders of rat and chick livers and fractions of such extracts are being studied with respect to the components required for activity.

Comparative absorption of amino acids from the intestine. ALINE UNDERHILL ORTEN, MARCEIL D. VANBUREN* AND CHARLES G. JOHNSTON.* *Dept. of Surgery, Wayne Univ. College of Medicine, Detroit, Mich.*

The absorption of individual amino acids from permanent Thiery loops during half-hour periods in dogs has been studied. The starch column chromatographic method has been used for separating and determining the amino acids in the intestinal loop contents. Results indicate that there is little difference in the individual absorption rates of D-L phenylalanine, L-leucine and D-L isoleucine from equimolar solutions. However, leucine, in the presence of either phenylalanine or isoleucine depresses the absorption of both phenylalanine and isoleucine. The comparative rates of absorption of the optical isomers of these amino acids are being studied.

Effect of whole-body x-irradiation on urinary B-vitamin excretion of rats. WILLIAM N. PEARSON,* JANET P. OWENS,* WILLIAM J. DARBY AND GRANVILLE W. HUDSON.* *Depts. of Biochemistry and Radiology, Vanderbilt Univ. School of Medicine, Nashville, Tenn.*

Mature female Sprague-Dawley rats, 200–225 gm. in weight, were fed a complete purified diet of casein, sucrose, fat, minerals and crystalline vitamins for a period of 2 weeks. Daily urinary excretion of riboflavin, niacin, folic acid, and the B_6 group was determined microbiologically. The rats were then subjected to 500 r of whole-body x-irradiation and daily urinary levels of the above vitamins determined. There was an immediate and pronounced rise in the urinary volume and in the excretion of these vitamins. Excretion levels were highest the day immediately following irradiation in the instance of folic acid, riboflavin and niacin. On this day niacin excretion was approximately twice, riboflavin output 3 times, and folic acid excretion was 6 times that of the controls. Excretion of the B_6 group was not maximal until the second day after irradiation and at that time the level was some 9 times that of the pre-irradiation control period. The vitamin excretion levels steadily decreased, reaching normal values from 3–5 days after irradiation. Experiments with pair-fed animals showed that the increased vitamin excretion was not due to decreased food intake. A discussion of the implications of these and additional data will be presented. (This paper is based on work performed under Contract with the United States Atomic Energy Commission.)

Effect of antibiotics on amino acid-supplemented rice diets. LOUIS J. PECORA (introduced by JAMES M. HUNDLEY). *Natl. Inst. of Arthritis and Metabolic Diseases, Natl. Insts. of Health, Bethesda, Md.*

It was reported earlier (PECORA AND HUNDLEY, *J. Nutrition* 44: 101, 1951) that a 90% rice diet could not be improved by the addition of lysine but marked growth resulted only when the combination DL-lysine HCl (2.00%) and DL-threonine (1.20%) was added. DL-Lysine HCl (0.32%) and DL-threonine (0.20%) gave growth response equal to the full supplements. Growth could not be further improved by the addition of other essential amino acids either singly or in groups of 2, 3 or 4. Antibiotics (20 mg.%) were added to many of the above diets which were fed *ad lib.* to groups of 5 littermate weanling rats for 9 weeks. Rats fed the unsupplemented 90% rice diet plus aureomycin or streptomycin gained 19.5 gm. ($p = < .05$) more than the controls. The bacitracin and penicillin group gained more than the controls though the increase was not statistically significant. Terramycin and chloromycetin were ineffective. The group fed the lysine supplemented rice diet plus terramycin grew 30 gm. ($p = < .01$) more than the controls, the other antibiotics gave no response. Rats fed the diet fully supplemented with lysine and threonine plus aureomycin gained 21 gm. ($p = < .05$) more than the controls while the other antibiotics were without effect. However improved growth response resulted when the smaller quantity of lysine and threonine was used plus certain antibiotics. The aureomycin fed group gained 45 gm. ($p = < .01$) and the penicillin fed group 30 gm. ($p = < .01$) more than the controls. Streptomycin and terramycin produced only a slight growth improvement, chloromycetin and bacitracin were ineffective. Food efficiency was 4.9 for the aureomycin group and 6.4 for the control.

Development of anemia on Newfoundland enriched flour. L. BRADLEY PETT. *Dept. of National Health and Welfare, Ottawa, Canada.*

Children in a boarding school were studied in 1948 and 1949 for a base line. Diets in each succeeding year were kept as comparable as possible, but all flour used since 1949 contained 3 added vitamins and iron according to U. S. standards, plus 500 milligrams of bone meal per pound of flour. Certified pipettes were used and hemoglobins measured photometrically. Children judged to have too low a hemoglobin value increased from the base line of 16–52%, in 1950, and 39% in 1951. The hemoglobin averages, and the skewness of the distribution curves corresponded. Iron administration to some cases supported the diagnosis of iron-deficiency anemia. In a control school in the same area anemia actually decreased, thus minimizing local or seasonal explanations. The study is still in progress, and observations are also being made regularly on other effects of enriched flour. If enriched flour caused the increase in anemia that was found, the explanation may rest in an interference with iron absorption by the calcium or phosphate in the added bone meal. The literature cites such possible results. If enriched flour did not cause the increase in anemia, the fact remains that no beneficial effect was observed from the iron in enriched flour. These results emphasize the danger of adding chemicals to foods on theoretical grounds without studying the physiological effects on humans, even though this is time-consuming.

Pantothenic acid deficiency in the guinea pig. MARY ELIZABETH REID. *Natl. Inst. of Arthritis and Metabolic Diseases, Natl. Insts. of Health, Bethesda, Med.*

Guinea pigs, supplied with a synthetic diet consisting of vitamin-free casein, sucrose, corn

starch, cellophane, corn oil, minerals and all the known vitamins except pantothenic acid, developed deficiency symptoms after periods of time which varied with the age at which the animals were placed on the diet—the younger the animals the earlier the onset. The most outstanding gross symptoms were a decrease in the growth rate followed by loss in weight, anorexia accompanied by inactivity and weakness, roughening of the coat, cyanosis at the margin of the ears and a tendency to have diarrhea. The internal deficiency symptoms were decrease in size of the visceral organs including the kidneys, liver, spleen and heart with enlargement of the adrenal glands and a gross decrease in mesenteric and subcutaneous fat. Some of these internal effects were undoubtedly due to inanition.

Collagen formation in vitamin A deficient rats. Wm. van B. Robertson and Valmore Cross (introduced by H. B. Pierce). *Univ. of Vermont College of Medicine, Burlington.*

Scurvy in susceptible species (primates and guinea pigs) characteristically results in an inability to lay down new collagen. The collagen concentration of forming subcutaneous fibrous tissue has been found to be a very sensitive measure of the status of ascorbic acid nutrition of guinea pigs. A similar system of collagen synthesis appeared well suited to test the suggestion of Mayer and Krehl (*Arch. Biochem.* 16: 613, 1948) that rats fed a synthetic diet deficient in vitamins A and C developed scurvy. Avitaminosis A presumably impaired normal synthesis of vitamin C by the rat. Irish moss extract was injected subcutaneously, inducing fibrous tissue formation in rats which were rendered 'scorbutic' by feeding the deficient diet of Mayer *et al.* and in rats receiving the same diet supplemented with either vitamin A or C. The collagen concentration determined by the method of Lowry *et al.* (*J. Biol. Chem.* 139: 795, 1941), was as high in the tissue from scorbutic rats as in that from the vitamin-supplemented rats. The ability of the deficient rats to form new collagen is in striking contrast to the evidence previously presented by Mayer *et al.*, which was so suggestive of scurvy. Possible implications for the problem of scurvy will be discussed.

Nutritional status of 60 women with respect to vitamin C. Charlotte Roderuck, Harriett Roberts,* Maxine Britton,* and Pearl Swanson. *Iowa State College, Ames, and the Bureau of Human Nutrition and Home Economics, Washington, D. C.*

Sixty women, ranging in age from 30–85 years and selected randomly in Ames, Iowa, served as subjects. During a 2-year period, 102 measurements of their vitamin C status were made, 5 women being observed 3 times, 32 women twice and 23 women once. Weighed food records were kept for 7 consecutive days and the nutrient intake calculated. Ascorbic acid concentrations of whole blood or serum and excretion of ascorbic acid in the urine for a 24-hour period both before and after a 400-mg. load dose were determined. After examination of the data, the following arbitrary standards for satisfactory vitamin C status were set up: 0.8 mg. ascorbic acid/100 ml. whole blood or serum, excretion of 15 mg. ascorbic acid/24 hours and excretion of 115 mg/24 hours following the load dose. Twenty-nine women met all these standards, 24 met none or only 1 and 7 were intermediate. When the intakes were examined, 5 of 29 women rated as having good vitamin C status ate less than 80 mg. of vitamin C daily while only 2 women rated poor in vitamin C status ate more than 80 mg. daily. Of 21 women excreting 215 mg. of ascorbic acid following the load dose, 15 were consuming more than 100 mg/day. Excretions of this magnitude approximate the 50% load dose return usually associated with tissue saturation.

Glutamine, a growth stimulating factor for *Lactobacillus gayoni* 8289. Dexter Rogers,* Agnar P. Nygaard,* Tsoo E. King* and Vernon H. Cheldelin. *Dept. of Chemistry, Oregon State Collge, Corvallis.*

Previous reports from this laboratory have reported the existence of a liver principle which was necessary for early initiation of growth of *Lactobacillus gayoni* 8289. This factor was not replaceable by any of the recognized B vitamins, including carnitine, protogen, or synthetic citrovorum factor. In a medium supplemented with xanthine, pyridoxamine, and extra amounts of sorbitan monooleate, KH_2PO_4, and yeast nucleotides (Cheldelin and Nygaard *J. Bact.* 61: 489, 1951), it has been found that L-glutamine possessed *L. gayoni* stimulatory activity. When graded levels of glutamine were compared with yeast extract, they furnished nearly equal stimulation, and the respective growth response curves were parallel up to optical densities of about 0.4. Glutamine was much less effective than could be accounted for on the basis of its content in yeast. Also, at optical densities of about 0.6, the growth with glutamine 'plateaued,' whereas the response to yeast extract remained a linear function of the logarithm of the yeast concentration up to optical densities of 0.7, or higher (30 mg. yeast extract/tube). On the basis of these and other observations, it appears that yeast extract contains a compound metabolically related to glutamine, which is essential for early optimum growth of *L. gayoni*.

Retention of essential amino acids when inadequate quantities are fed to the growing chick. WALTER C. RUSSELL, M. WIGHT TAYLOR AND WILLIAM A. PRICE, JR.* *Dept. of Agricultural Biochemistry, New Jersey Agricultural Experiment Station, Rutgers Univ., State Univ. of New Jersey, New Brunswick.*

When a diet containing animal protein sources of high nutritive value (dried whole beef, dried beef liver, dried egg, and fish meal) provided adequate amounts of histidine, isoleucine, lysine, threonine, and valine, but 9–30% less than the recommended allowances for arginine, leucine, methionine, pheylalanine, and tryptophane, and a protein level of 16.3% instead of the frequently used 20%, the retention of essential amino acids by the growing chick ranged from 39–71%. Fifty-six% of the total nitrogen was retained and 38% excreted. In an earlier experiment, using a starter mash, in which an adequate quantity of each of the essential amino acids was supplied and the protein content was 21.9%, the retention of the essential acids ranged from 27–43%; the total nitrogen retained was 33% and that excreted 60%. Growth of the chicks which consumed the diet containing proteins of high nutritive value and feed efficiency were equal to those for chicks fed a starter mash. It is significant that on a diet which provided 5 essential amino acids and total protein at less than accepted values, growth and feed efficiency were considered normal and the utilization of essential amino acids was more efficient than on a starter mash.

Goitrogenic action of excess calcium and of propylthiouracil alone and in combination in adequate synthetic diets. MYRA M. SAMPSON AND ANN PUTZKI.* *Dept. of Zoology, Smith College, Northampton, Mass.*

Female albino rats were isolated at the age of 25 days and fed for 45 days on 1 of 4 adequate synthetic diets of equal calorie value containing Borden's Sodium Caseinate, with or without inclusion of either or both of the following: an addition of 2% calcium carbonate and 0.25% propylthiouracil. Paired feeding technique was employed with sets of 3 rats in 2 series of experiments conducted simultaneously, using adequate calcium diets with or without the drug in 1, and excess calcium diets with or without the drug in the other series. The average percentage thyroid weights in mg. were approximately the same in calcium adequate intake-unlimited and weight-paired controls; 4 times greater in both calcium excess controls; 10 and 15 times greater respectively in rats fed drug-containing adequate and excess calcium diets. Histological study indicates that excess calcium or propylthiouracil induce hyperplastic goiter and the 2 combined lead to more extreme goiter than either alone. A striking difference in the effect of excess calcium as compared with that of propylthiouracil is the high incidence of mitosis in thyroids of intake-unlimited controls on the excess calcium diet, and the similar incidence in rats on the excess calcium diet containing the drug. Few or no mitoses were observed in thyroids of rats on any of the other diets. Further experiments are in progress. (Supported by research grant from the George H. Hood Foundation.)

Elimination of interference in determination of ascorbic acid by the 2,4-dinitrophenylhydrazine methods. VICTOR SCHOCKEN* AND JOSEPH H. ROE. *Dept. of Biochemistry, George Washington Univ. School of Medicine, Washington, D. C.*

Interference is sometimes experienced in the determination of ascorbic acid and certain of its derivatives by the methods involving the coupling of these compounds with 2,4-dinitrophenylhydrazine. The interference arises from the presence of enol-tartronaldehyde and similar compounds which have come to be known as 'reductones.' These substances are found in appreciable quantities principally in processed foods. In the present work it is shown that correction can be made for the presence of reductones in 2 ways: *1*) The reductones will couple with the 2,4-dinitrophenylhydrazine in 18N sulfuric acid at room temperature; ascorbic acid, dehydroascorbic acid, and diketogulonic acid do not form derivatives with 2,4-dinitrophenylhydrazine under these conditions. *2*) The absorption spectrum of the ascorbic acid chromogen is sufficiently different from that of the reductone derivative to permit calculation of the concentration of each in the presence of the other.

Sulfur-containing amino acids and vitamin E in dietary necrotic liver degeneration. KLAUS SCHWARZ (introduced by FLOYD S. DAFT). *Natl. Inst. of Arthritis and Metabolic Diseases, Natl. Insts. of Health, Bethesda, Md.*

When dietary necrotic liver degeneration is produced in rats by a vitamin E free 30% torula yeast ration, growth is impaired after 3 to 4 weeks. The average survival time is 45.5 days. For protection against liver necrosis DL- or L-methionine showed only 35% of the activity of L-cystine, when compared at molar equivalent dosage levels (10 rats/group). This ratio was suggestive of an inadequacy in the transformation of methionine to cystine. DL-Homocystine had activity equivalent to methionine. It was found in repeated experiments that *L*-cysteine when mixed into the diet or when fed by stomach tube was as ineffective as methionine and homocystine. It appears that the oxidation of cysteine to cystine—the last step in the methionine-cystine transformation—

is insufficient under these conditions. Vitamin E did not influence the growth response to cystine but did affect the utilization of cysteine. When supplementation with .5% cysteine was started after a depletion period of 24 days on the basal ration, animals receiving vitamin E grew steadily at an average of 6.4 gm/week, whereas animals without vitamin E averaged only 3 gm/week for the first 3 weeks and then completely stopped growing with an average body weight of 89 gm. The relation of 'factor 3,' a new factor against dietary necrotic liver degeneration obtained in purified form (*Proc. Soc. Exper. Biol. & Med.* 178: 852, 1951), to vitamin E and to cystine will be discussed.

Utilization of lysine from foods by the protein-depleted rat. B. S. SCHWEIGERT, BARBARA T. GUTHNECK* AND BARBARA A. BENNETT.* *Div. of Biochemistry and Nutrition, American Meat Inst. Fndn. and Dept. of Biochemistry, Univ. of Chicago, Chicago, Ill.*

Experiments were conducted with protein depleted adult rats to devise suitable conditions for the quantitative evaluation of the utilization of lysine when supplied by foods processed in different ways. A basal ration composed of purified amino acids (without lysine) and all other known nutrients has been used after depletion of animals that had been fed a purified ration without added protein. After repletion and subsequent depletion, the same animals were successfully used in a repeat experiment. Known amounts of lysine were added in the form of purified proteins or as foods (determined microbiologically) and the weight recovery, food efficiency, and for certain groups, the lysine excreted in the feces by the protein-depleted rat, were determined. The weight gains when graded levels of lysine were fed were compared with those from groups fed different foods as a source of lysine. The utilization of lysine was calculated from the above data and ranged from 65–90% for the different test products. Small amounts of lysine were excreted in the feces when lysine was fed as beef round, lactalbumin or casein (less than 5% of that ingested) while somewhat larger amounts were excreted when lysine was fed as soybean meal, wheat germ or peas (12–22% of that ingested). These studies are being extended with the use of a basal ration containing sesame meal plus supplements of certain amino acids.

A simple microbiological nutritional assay procedure employing seeded agar tubes. MAURICE E. SHILS, ANNA C. GELMAN* AND MARTIN HOROWITZ*. *Columbia Univ., School of Public Health, New York City.*

The diffusion principle of the agar plate assay for vitamins has been adapted to a capillary tube assay capable of detecting minute amounts of nutrients simply and quickly. The glass tubes, 80 mm. in length and 2 mm. in inside diameter are partially sealed at 1 end and filled by immersion to about $\frac{2}{3}$ of the length with an agar-basal medium seeded with the test organism. The basal medium, deficient only in the nutrient being measured, is autoclaved after the addition of 0.6% agar. Five ml. of bacterial suspension of the proper turbidity are then added at 42°–45° C. and the tubes filled and allowed to cool. The test material is added above the agar column with a constriction micropipet in volumes ranging from 0.015–0.060 ml., and the tubes are incubated, without sealing, in a well-humidified chamber for 16–20 hours at the temperature optimum for the test organisms. The organisms in the agar which obtain the critical nutrient in its diffusion downward multiply and the zone of growth is well-defined by its cloudiness contrasting with the clear appearance of the seeded agar without growth. The length of the columns of growth are measured with a simple vernier caliper or other measuring device. Using *Lactobacillus fermenti* 9338 in testing for thiamine, *L. casei* 7469 for riboflavin, and *L. arabinosus* 8014 for niacin and pantothenic acid, we have found the lengths of the column of growth (in mm.) to bear a linear relation to the logarithms of the concentrations of vitamin added to the tubes over much of the range tested. Data will be presented on a number of variables which have been tested and on the results obtained when testing the vitamin content of various biological materials.

Influence of processing on efficiency of proteins in cottonseed and soybean meals. BARNETT SURE. *Dept. of Agricultural Chemistry, Univ. of Arkansas, Fayetteville.*

Various cottonseed and soybean meals prepared by different processes were fed at 10, 8, and 5% protein levels to albino rats during an experimental period of 10 weeks and the results were calculated as gains in weight/gm. of protein intake. There were 24 animals in each group, 12 males and 12 females. Four types of cottonseed meals were used, 3 of which were prepared by the Protein Division of the Southern Regional Laboratories, as follows: 1—organic-solvent extracted (OSE) and 2 prepared by expeller processes. The fourth was a commercial product (PROFLO). The OSE product proved the best and PROFLO the poorest from the standpoint of protein efficiency. Two commercial soyean meals were used and 1 commercial soybean flour (NUTRISOY). The latter proved the poorest from the standpoint of protein efficiency, because of losses in methionine during commercial processing. The addition of 0.2% *dl*-methionine to the rations containing NUTRISOY doubled the protein efficiency on a

10% protein level and trebled the protein efficiency on the 8% protein level.

Food habits of women 30–90 years old. PEARL SWANSON, MARGARET OHLSON, LIDA BURRILL*, ALICE BIESTER*, JANICE SMITH AND ESTHER BATCHELDER. *State Colleges of Iowa, Ames, Michigan, East Lansing, and North Dakota, Grand Forks, Univ. of Minnesota, Minneapolis and Illinois, Urbana, and Bureau of Human Nutrition and Home Economics, Washington, D. C.*

A study of food habits, and how they change with the progression of age, in individuals living in their usual home environments is basic to investigations designed to establish dietary standards for aging people. In this report, an analysis of the food habits of 1072 Iowa women selected on the basis of an area sample will be presented. Records used were 1-day dietary recalls. The data show that the average energy value of the daily diet is 1700 Calories; its average protein content, 57 gm. Eighty-six per cent of the women consume less than 2400 Calories daily; 60% less than 1800 Calories. Sixty per cent of the women eat less than 60 gm. of protein daily. With each advancing decade, mean daily intakes of all nutrients decrease. The respective roles that the energy-producing foods and the proteins play in determining the concentrations of other nutrients in the diet will be illustrated. The apparent health of the women is being correlated with their intakes of energy foods and of protein. Finally, the Iowa data will be compared with those obtained in similar surveys of groups of women living in South Dakota, Minnesota, Michigan and Illinois. In all, approximately 2500 subjects will be represented in the study. (North Central Cooperative Study (NC-5) on Nutritional Status and Dietary Needs of Population Groups.)

Interrelationships between choline and vitamin B_{12} in the rat and in the mouse. JOHN J. TRAVERS* AND LEOPOLD R. CERECEDO. *Dept. of Biochemistry, Fordham Univ., New York City.*

Rapid and uniform depletion of the vitamin B_{12} stores of the livers of rats was accomplished with a diet containing sulfasuxidine and iodinated casein, but free of vitamin B_{12} and choline. The growth of these rats was stimulated by the administration of vitamin B_{12}, and of choline. Vitamin B_{12} was found to be without effect if choline was present in the diet at an adequate level. Under the conditions used, choline, supplied in the diet, prevented to a large extent the depletion of the liver stores of vitamin B_{12}. The choline content of the kidneys of choline deficient rats was significantly lower than that of choline fed rats, and was not affected by the administration of vitamin B_{12}. Under similar conditions, different results were obtained with mice. The administration of choline had no effect on the vitamin B_{12} content of the livers of mice, nor on the choline content of the kidneys of mice. (This investigation was supported in part by a grant from the National Cancer Inst., Natl. Inst. of Health, Public Health Service.)

Carbohydrate tolerance in obese and non-obese subjects. THEODORE B. VAN ITALLIE,* RACHEL BEAUDOIN* AND JEAN MAYER. *Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.*

Studies on food intake in animals suggest that blood sugar gradients may play an important role in the regulation of appetitite. Carbohydrate tolerance in obese and nonobese women, paired with respect to age and height, was studied. Values for capillary and venous blood sugar and serum inorganic phosphorus were determined before and at 15-minute intervals for an hour following *a*) ingestion of a self-selected meal, and *b*) after ingestion of a solution containing 50 gm. of glucose. Prior to the experiment, these subjects were asked not to modify their usual food habits. The tests were done at the noon hour. In every case a careful dietary record was obtained for the day of the test and the preceding day. Tolerance curves showed a wide spread in the obese and nonobese categories. Both groups contained a few members who responded to the ingested carbohydrate with an exaggerated increase in blood sugar which fell outside the normal range. In the majority of cases, a striking discrepancy in carbohydrate tolerance between obese and nonobese subjects was observed. Characteristically, the obese group showed a sluggish rise in blood sugar which was ⅓–½ that of the nonobese group during the first ½ hour. The differences between the 2 groups were particularly significant during the first ½ hour when glucose was given, and during the second ½ hour after the self-selected meal. Changes in blood inorganic phosphorus and capillary-venous differences in levels of blood sugar were not remarkable. The possible significance of these findings will be discussed.

Effect of pantothenic acid deficiency and food intake on respiration of duodenal mucosa. JOSEPH J. VITALE* AND D. M. HEGSTED. *Dept. of Nutrition, Harvard School of Public Health, Boston, Mass.*

The change in the respiration of duodenal mucosa during the development of pantothenic acid deficiency was studied. Weanling rats were fed a purified diet lacking pantothenic acid. Q_{O_2} remained normal, average 14, during the first 5 weeks and then gradually fell to approximately 6. *Ad libibtum* fed controls remained at the normal level. Control animals restricted to 10 gm. of diet/day also showed a decrease in respi-

ration of the mucosa similar to that seen in deficient animals. The ration fed these semistarved animals was then changed to a high high protein-high vitamin ration. Although food intake remained limited, the Qo_2 of the duodenal mucosa returned to normal in approximately 10 days. It appears that both the semistarved and pantothenic acid-deficient animals were being deprived of an essential or essential nutrients other than pantothenic acid. The effect of diets of various composition on the respiration of duodenal mucosa will be reported.

Determination of unsaturated fatty acids in blood serum. HILDA F. WIESE* AND ARILD E. HANSEN. *Dept. of Pediatrics, Univ. of Texas Med. Branch, Galveston.*

A semimicro method for the determination of unsaturated fatty acids in blood serum by spectral analysis has been devised. One objection to the application of the current spectral methods for 2, 3, 4, 5 and 6 double bond fatty acids to human serum is the large amount of fatty acid (100 mg.) required for the analysis. By means of a modified procedure for conjugation of the fatty acids, it is possible to determine the above unsaturated fatty acids in 3 ml. of serum. The essential steps in the semimicro procedure consist in conjugating 7–15 mg. of purified fatty acid with 2 ml. of 11% KOH in ethylene glycol at 180°C. for 25 minutes. Spectral densities are read in methyl alcohol solution at 2350Å, 2700Å, 3000Å, 3475Å and 3750Å on a Beckman D. U. spectrophotometer. Using known mixtures of linoleic, linolenic and arachidonic acid, varying in concentration from 4–32%, with different amounts of palmitic and stearic acid, the recovery with few exceptions was within 5% of the theoretical amount of the unsaturated fatty acid present. Neither linoleic nor linolenic acid showed absorption above their respective maximum wave length. Arachidonic acid showed slight absorption at 3475Å but none at 3750Å. Results by the semimicro method have been compared with those by the Holman and Burr macromethod using 3 dietary fats, dog serum and rat tissues. Absorption at 3475Å and 3750Å occurred less frequently by the semimicro method than by the macromethod. Higher percentages of 2 double bond fatty acids were found by the former method. (Support for this work was received by a contract sponsored by the Bureau of Human Nutrition and Home Economics, U. S. Dept. ef Agriculture and authorized by the Research and Marketing Act.)

A rapid simplified microbiological assay method for vitamin B_{12} and other vitamins. WILLIAM L. WILLIAMS, RAYMOND G. ESPOSITO AND JACK V. PIERCE (introduced by T. H. JUKES). *Lederle Labs. Division, American Cyanamid Co., Pearl River, N. Y.*

The vitamin B_{12}-requiring *E. coli* mutant, number 113-3 (DAVIS AND MINGIOLI, *J. Bact.* 60: 17, 1950) has been utilized for the assay of vitamin B_{12} by means of a new rapid simplified technique. Test solutions are pipetted on filter paper discs and the discs are placed on the basal medium solidified with agar. Zones of growth are measured at any time from 12 hours to 3 days. The basal medium is simple and chemically defined consisting of inorganic salts and glucose. Pads containing known amounts of vitamin B_{12} can be prepared in advance so that standard curves can be prepared in a few minutes. Aseptic conditions are not essential. One-third or less time is required to carry out the pad-plate procedure compared to the test-tube technique. The method has the fundamental advantage that vitamin B_{12} and vitamin B_{12b} as well as ammino-cobalamin, nitroso-cobalamin and sulfite-treated cyano-cobalamin all have equal activity. Relatively large amounts of methionine will give growth of the organism in the absence of vitamin B_{12}. This interference can be readily detected in the pad-plate method by the faint diffuse growth zones exhibited by methionine. The pad-plate has also been used successfully for the assay of protogen with *Streptococcus faecalis* by reversal of propionate inhibition, for the assay of LBF with *Lactobacillus bulgaricus*, and for the assay of 'citrovorum factor' with *Leuconostoc citrovorum*. Preliminary experiments with the yeast, *Saccharomyces carlsbergensis* indicate that vitamin B_6, pantothenic acid, inositol and biotin can be assayed with this organism using the pad-plate procedure.

Evidence for a metabolic function of thiamine not mediated through cocarboxylase. D. W. WOOLLEY AND R. B. MERRIFIELD.* *Rockefeller Inst. for Med. Research, New York City.*

The signs of thiamine deficiency have usually been attributed to a lack of cocarboxylase in metabolic reactions, with consequent elevation of pyruvate levels in the tissues. The following evidence suggests that the classical signs of the avitaminosis may be due to an unrecognized function of thiamine not concerned with cocarboxylase or with elevated tissue pyruvate. Manifestations of thiamine deficiency in animals can be produced by administration of either pyrithiamine (neopyrithiamine) or oxythiamine. In both cases the vitamin will prevent or cure the disease elicited by the analog. However, the signs usually attributed to neurological disturbances are seen only with pyrithiamine (neopyrithiamine). With oxythiamine a less typical syndrome is called forth, but, as others have shown, it is characterized by diminished cocarboxylase in the

tissues, and by elevated blood pyruvate. With pyrithiamine (neopyrithiamine) however, the levels of cocarboxylase in the liver, and of pyruvate in the blood, are normal, even as the animal is succumbing with typical manifestations of thiamine deficiency. While it is possible *in vitro* to prevent the enzymatic synthesis of cocarboxylase from thiamine with pyrithiamine (neopyrithiamine), the amounts needed are large compared to the lethal dose for an animal. It would thus appear that the analog, in calling forth characteristic manifestations of thiamine deficiency, interferes primarily with a function of thiamine responsible for the typical neurological signs, and that this function is distant from the one involving cocarboxylase and pyruvate. Attempts are being made to elucidate this postulated new function of thiamine.

AMERICAN ASSOCIATION OF IMMUNOLOGISTS

Thirty-sixth Annual Meeting

NEW YORK CITY, APRIL 14–18, 1952

(An asterisk following an author's name indicates "by invitation")*

Relation of herpes virus to mitochondria of host cells. W. WILBUR ACKERMANN (introduced by THOMAS FRANCIS, JR.). *Dept. of Epidemiology and Virus Lab., Univ. of Michigan, School of Public Health, Ann Arbor.*

A relation has been established between certain phases of metabolism localized in the mitochondria of host cells and the propagation of herpes and influenza viruses. The concept that these cellular organelles function in the development of virus is supported further by this report wherein is described a physical association of the virus with the mitochondria. Livers infected with herpes virus were removed from 14 day old embryonate eggs. A 20% homogenate was prepared in 0.25 M sucrose using the Potter-Elvehjem-glass homogenizer. Fractions composed of nuclei, mitochondria, submicroscopic particles and supernatant fluid were obtained by differential centrifugation as described by Schneider and Hogeboom (1950). Subsequent viral titrations revealed a considerable activity in all fractions as reported previously by Francis and Kurtz (1950). An attempt was made to determine whether this viral activity was a superficial contamination of the particulate fractions. The mitochondria were washed repeatedly in cold isotonic sucrose. After each washing the residue was sedimented under conditions of time and centrifugal force which do not sediment the virus. Titrations of the virus in the mitochondria after each of 5 successive washings revealed a constant titer by the second washing. About 10% of the virus is associated with the mitochondria in what is more than a superficial attachment. Mitochondria prepared from normal liver were suspended in a supernatant fluid obtained by homogenizing and centrifuging infected tissue. Using the procedure described above, the mitochondria were readily separated from the virus indicating that under these conditions there was no significant union of virus and mitochondria. Approximately 90% of the virus appears to exist as free virus unattached to particulate materials of the cell. A significant part is so firmly attached to the mitochondria that it is not removed by successive washings in cold isotonic sucrose.

Effect of urea on antigen-antibody precipitation reactions. JAY BANOVITZ (introduced by DAN H. CAMPBELL). *Gates and Crellin Labs. of Chemistry, California Inst. of Technology, Pasadena.*

In view of the possible significance of hydrogen bonding as one of the forces involved in antigen-antibody reactions, an investigation has been undertaken to quantitatively study the effect of a hydrogen bonding material, such as urea, on specific precipitation. Two systems, namely, bovine serum albumin and arsanilic azo dyes will be discussed. Results obtained so far are in essential agreement with the qualitative data obtained by Landsteiner and Welecki (*Z. Immunitätsforsch.* 8: 397–403, 1911) who found that urea concentrations up to 2 molar had little effect. Effects obtained at higher concentrations will be discussed in relation to antibody denaturation and specific combination of antigen-antibody combining sites.

Neutralizing antibodies in mumps. WINSLOW J. BASHE, JR.,* TAMAR GOTLIEB,* GERTRUDE HENLE* AND WERNER HENLE. *Children's Hosp. of Philadelphia and the Univ. of Pennsylvania, Philadelphia.*

An intra-allantoic neutralization test (ENT), using varying dilutions of serum and 1000–10,000 ID_{50} of virus has been developed and standardized. While the ENT with convalescent sera usually gave high titers when complement fixation (CF) and hemagglutination inhibition (HAI) were also high, on rare occasion one or the other of the respective antibodies remained at low levels or negative. In sera from random individuals the neutralizing antibodies were, as a rule, absent when history and CF were negative and the ENT was usually positive when a past history and CF antibodies were recorded. However, a few individuals were encountered with positive ENT and negative CF and conversely. Both ENT and CF, with few exceptions appeared to yield reliable information as to the immune status in this group of individuals. Following subcutaneous or intradermal vaccination with inactivated virus, or oral spraying with active attenuated virus, neutralizing antibodies developed with varying frequency and to varying height. If the CF response was low

the ENT frequently remained negative. Correlation of the data with the incidence of mumps 9–12 months following immunization indicated a more significant relationship between immunity acquired by vaccination and ENT than with CF or HAI.

In vivo effect of x-irradiation on guinea pig complement. C. Cooper Bell, Jr. (introduced by William H. Taliaferro). *Univ. of Chicago, Chicago, Ill.*

The quantitative effect of 100, 150, 200 and 600r on guinea pig complement (50% hemolytic units determined photometrically) has been studied. Male and female animals, selected at random, weighing between 550–850 gm., were bled from the heart. Serums from individual guinea pigs were titrated for complement 1 day before ½, 1, 1½, 2, 3, 4, 5, 6, 7, 8, 9 and 10 days following irradiation. Serums from normal guinea pigs were titrated at the same time to determine the effect of bleeding per se. Eight-tenths ml. of blood could be taken from the same animal at least 4 times. Data from 5 or more guinea pigs were pooled to obtain the statistical differences. The 100r group showed a 17% drop in complement titer at 24 hours ($P = 0.019$), recovery by 5–6 days, a 12% drop at 7 days ($P = 0.025$) and beginning recovery at 10 days if the animal survived. The 150 and 200r groups followed the same general pattern ($P = 0.001$ for 200r at 24 hours). The titers of the 600r group were less than the control group throughout—with all P values less than 0.05. The 24 hour drop, however, was no larger than that of the 100r group. There was a 46% drop at 10 days.

Pathogenesis of poliomyelitis in normal and passively immunized primates after virus feeding. David Bodian. *Johns Hopkins Univ. Dept. of Epidemiology, Baltimore Md.*

Passive immunization at the time of virus feeding was used to assess the role of antibody per se in immunity, and to eliminate viremia as a factor in pathogenesis. The effect of passive antibody on paralysis was studied in chimpanzees and in cynomolgus monkeys; its effect on virus excretion in feces and on the serum antibody response to virus feeding was studied in chimpanzees. Passive serum antibody levels of 1:100 to 1:6 at the time of virus feeding prevented paralysis in 73 monkeys; 10 of 65 controls became paralysed. Virus was isolated from blood of 2 of 3 control chimpanzees in the pre-febrile period, just before a sharp serum antibody rise (from 0 to 1:1000 in 2 days), and 2 and 5 days before onset of paralysis. Serum antibody was at 1:1000 at onset of paralysis in both instances. A passive serum antibody level of 1:500 apparently prevented paralysis and viremia in 3 chimpanzees but delayed and reduced fecal virus excretion occurred in 2 during the third week, followed by an active antibody response. This response was lower than in the controls, but persisted at 1:100 10 weeks after induction of a passive antibody level of 1:500. Since the half-life of the passive antibody (rhesus hyperimmune serum) was found to be 12 days, the expected level at this time was about 1:10. It appears *1*) that viremia may occur early in the course of infection by the alimentary route; *2*) that low levels of passive antibody can prevent paralysis following virus feeding; *3*) that fecal virus excretion can occur without an antecedent viremia, and in the presence of levels of serum antibody of the order of 1:100; *4*) that a delayed active antibody response can occur under these conditions, presumably as a result of virus multiplication in the alimentary tract, but at a lower level than occurs in the unmodified infection.

Chemical modification of a natural plant agglutinin. William C. Boyd. *Boston Univ. School of Medicine, Boston, Mass.*

A number of plants, especially legumes, contain naturally occurring agglutinins for human and other red blood cells. Some plants, especially Lima beans, contain agglutinins specific for the A antigen of groups A and AB. The effect of various chemical modifications has been investigated. Treatment over night with N/10 NaOH destroyed the agglutinating activity, as did treatment with 0.4% formaldehyde. Various amines were diazotized and coupled to the plant globulin in various proportions. Considerable amounts of aniline, para-amino-benzoic acid, arsanilic acid, and other acid aromatic amines could be introduced into the protein molecule without causing any noticeable alteration in the agglutinating titer. Treatment with a large excess often destroyed the agglutinating activity, perhaps merely from the effects of the large amount of strong chemical reagents which had to be used. Coupling of diazotized trimethyl-amino aniline, which introduces positively charged groups into the molecule, also did not affect the activity except in large amounts. From these preliminary experiments it would seem that the amino groups of the protein are not prominently concerned in its reaction with the blood group substance A.

Phagocytosis of normal, sensitized and trypsinized erythrocytes by tissue culture macrophages. Noreen G. Brandt,* Joseph A. Bass,* Matthew C. Dodd and Claude-Starr Wright.* *Ohio State Univ., Depts. of Medicine and Bacteriology, Columbus.*

The following observations on normal, sensitized and enzyme treated erythrocytes in tissue culture were made as a further investigation of the immuno-hematological aspects of these cells.

The study was carried out using macrophages cultured from rabbit spleens in the presence of homologous serum. Erythrocyte suspensions and test sera were added simultaneously after removal of excess fluid. Routine observations were made after 20 minutes incubation. The normal rabbit erythrocyte in the presence of homologous or heterologous serum was not phagocytized but in the presence of specific immune serum phagocytosis occurred. Preliminary observations were made on the phagocytosis of erythrocytes by macrophages from sensitized rabbits. Treatment of the erythrocyte with trypsin likewise prepared it for phagocytosis by macrophages from normal animals. Similarly, trypsin treatment also promoted phagocytosis of the human erythrocyte which normally was not phagocytized in the presence of rabbit serum. Normal human serum has an inhibitory effect on the phagocytosis of trypsinized human and rabbit erythrocytes which can be removed by heating at 56° for 30 minutes, by absorption with rabbit spleen and by dilution. The inhibitory factor is not lost on storage. The phagocytosis of rabbit erythrocytes in the presence of hyperimmune serum can be correlated to some extent with agglutinin titers. Both rabbit anti-human erythrocyte serum and human anti-A serum, although they caused agglutination, failed to promote phagocytosis of normal human erythrocytes.

Effect of hyaluronidase on the reaction of rabbits to intradermal inoculations of vaccinia virus. B. A. Briody and F. Duran-Reynals.* *Dept. of Microbiology, Yale Univ. School of Med., New Haven, Conn.*

Ten-fold serial dilutions of 'calf lymph' vaccinia virus were made in saline, and each dilution divided into 2 portions. To one portion was added an equal volume of saline, and to the other an equal volume of 1% commercial hyaluronidase or a 10% extract of rat testes. These 2 sets of virus dilutions were then inoculated intradermally in non-immunized rabbits, in rabbits passively immunized with hyperimmune anti-vaccinia rabbit serum, and in rabbits that lost some or most of an actively acquired immunity to vaccinia virus. The saline series of virus dilutions were injected on the right side, and the hyaluronidase or testicular extract series on the left side of the rabbit. The data obtained indicate that the lesions induced by all dilutions of virus in the non-immunized and the passively immunized rabbits were enhanced by hyaluronidase or testicular extract when contrasted with the lesions occurring in the saline series of dilutions in the same rabbits. On the contrary, in the rabbits with a partial active immunity, the lesions induced by the virus beyond a certain, critical concentration are not enhanced or frankly suppressed by the spreading effect of hyaluronidase. When the reactions of the rabbits are expressed in terms of the ratio of the diameter of the lesions of the saline series to the diameter of the lesions of the hyaluronidase or testicular extract series, the ratios are 0.6, 0.6, and 1.1 for the non-immunized, the passively immunized, and the partially (active) immune groups of rabbits respectively.

Influence of chemicals on propagation of poliomyelitis in tissue culture. Gordon C. Brown. *Dept. of Epidemiology and Virus Lab., Univ. of Michigan, School of Public Health, Ann Arbor.*

Two methods of culturing virus have been used, the flask method with human embryonic material as described by Enders, Weller, and Robbins, and the roller tube method with monkey testicle tissue. Three immunologically identical strains of Lansing type poliomyelitis virus, Lansing, YSK and MEF-1, grew by both methods, but marked strain differences were noted. The MEF-1 strain, which had been previously adapted to suckling mice, produced consistently higher titers in the fluids as measured by mouse infectivity tests and cell injury. Therefore, this strain and monkey testicle tissue by the roller tube technique was selected as the method of choice for testing the majority of the chemicals. A wide variety of compounds including amino acid and growth factor analogues have been tested. Most of these had little or no effect on virus growth. Certain antimetabolites, however, suppressed markedly the growth of virus in tissue culture. Some of these apparently interfered with the synthesis of virus without affecting the cells, while others appeared to act directly on the virus or upon the cells rendering them incapable of supporting virus growth. Even though many of these compounds have failed to influence the course of poliomyelitic infection in mice, this method of testing is useful in acquiring information about the growth requirements of this virus.

Inhibition of bacterial esterase activity by polymyxin B. Sidney Cohen, C. V. Purdy and J. B. Kushnick (introduced by M. J. Schlesinger). *Dept. of Labs., Beth Israel Hosp., and Dept. of Medicine, Harvard Univ. Med. School, Boston, Mass.*

Commercial preparations of polymyxin B have been found to inhibit markedly the esterase activity of several species of mycobacteria when suspended in solutions of low salt concentration. The esterase activity of *M. butyricum*, *M. phlei*, and *M. tuberculosis* (H37Ra and BCG strains) has been studied with tributyrin and with the synthetic substrates beta naphthyl acetate and beta naphthyl laurate. Washed bacteria or soluble extracts were preincubated with aqueous solu-

tions of antibiotics. The suspension of substrate in buffer was then added and the degree of hydrolysis measured either colorimetrically (SELIGMAN *et al. Am. J. Physiol.* 159: 337, 1949) or manometrically. Esterase activity of whole bacteria and of extracts was markedly inhibited by 2 preparations of polymyxin B of 60 and 75% purity. Hydrolysis of naphthyl laurate by *M. butyricum* was 90% inhibited by 17.5 μg polymyxin/ml. and 50% inhibited by 1.75 μg polymyxin/ml. In the presence of 0.07 M NaCl solution, the inhibitory effect of polymyxin upon esterase activity was greatly reduced. Chloramphenicol, streptomycin and penicillin had little or no effect on bacterial esterase activity. An analogous effect of electrolytes upon a biochemical reaction of polymyxin B has been reported by Latterade and Macheboeuf (*Ann. Inst. Pasteur* 78: 753, 1950) who found that precipitation of acidic substances such as ribonucleic acid by polymyxin B was inhibited by electrolytes.

Tuberculous allergy induced in humans with chemical constituents of tubercle bacillus. NINE CHOUCROUN. *Cornell Univ. Med. College, Dept. of Public Health and Preventive Medicine, New York City.*

In previous reports we have shown that tuberculin type of hypersensitivity can be induced in normal animals by components extracted from the tubercle bacillus. Tuberculous allergy was established first in normal guinea pigs with a paraffin oil extract of dead tubercle bacilli (*Compt. Rend. Acad. d. Sc.* 208: 1757, 1939), then with components separated from the extract (*Science* 98: 327, 1943). Among these components the lipopolysaccharide Pmko, ester of mycolic acid, was shown to be not only an antigenic component with regard to carbohydrate antibodies (*Am. Rev. Tuberc.* 56: 203, 1947) but also the component responsible for the establishment of the tuberculin type of hypersensitivity (*Compt. Rend. Acad. d. Sc.* 226: 1477, 1948; *Tuberculologie* November, 1949). The essential role played by this lipopolysaccharide in determining hypersensitivity has been recognized recently by Raffel (*Experientia* VI: 413, 1950). When injected together with tuberculoprotein this lipid component allows the development of a state of hypersensitivity revealed by a strong positive reaction to the standard tuberculin test. As small an amount as 100 gamma of each component given, intraperitoneally, twice at fortnightly intervals in 1 cc. paraffin oil is enough to bring about this response. The same result has been obtained with humans previously negative to the standard tuberculin test. After receiving a single subcutaneous injection of 0.2 cc. paraffin oil containing 250 gamma of each component, 5 adults and 9 children so immunized responded 5 weeks later with a positive reaction to the same standard tuberculin test. One of them, 22 years of age, was still strongly positive 27 months after immunization.

Persistence of C^{14}-anthranil-azo-ovalbumin in rabbits. CHARLES F. CRAMPTON,* HERBERT H. REILLER* AND FELIX HAUROWITZ. *Dept. of Chemistry, Indiana Univ., Bloomington.*

Anthranil-azo-ovalbumin (AAO) was prepared by diazotizing anthranilic acid (0.1 g.) containing C^{14} in the carboxyl group and coupling it with ovalbumin (0.9 g.). Rabbits intravenously injected with 30 mg/kg. AAO were killed at intervals from 5 minutes to 148 days after injection. Their organs and subcellular fractions, isolated from liver and spleen by differential centrifugation, were analyzed for trichloroacetic acid precipitable radioactivity. Like iodo-ovalbumin (*Federation Proc.* 10: 560, 1951), AAO is cleared rapidly from the blood and deposited in the organs, particularly the bone marrow. During the first minutes following injection AAO is concentrated in the microsomes and mitochondria of liver and spleen, but later it is concentrated only in the mitochondria. The liver of a rabbit killed 148 days after injection contained 0.0045 mg. AAO ($\approx 6 \times 10^{13}$ molecules), the bulk of which was found in the mitochondrial fraction. AAO added *in vitro* to homogenates of normal rabbit organs was not adsorbed to the granules, but was recovered in the soluble supernatant fraction. Intravenously injected anthranilic acid was not appreciably retained. Our results are in agreement with the findings of MacMaster and Kruse (*Federation Proc.* 10: 564, 1951) on the persistence of azoproteins in mice. The fact that a significant number of antigen molecules is stored indefinitely in the cytoplasmic granules is consistent with the view that the presence of antigen molecules is a prerequisite for the formation of antibody. (Supported by the Office of Naval Research, the U. S. Atomic Energy Commission, and the U. S. Public Health Service.)

Estimates of particle number, lethal effect and chemical properties of virus of meningopneumonitis. T. TIMOTHY CROCKER (introduced by KARL F. MEYER). *Div. of Medicine and Hooper Fndn. for Med. Research, Univ. of California School of Medicine, San Francisco.*

In the course of concentration and purification of elementary bodies of the virus of meningopneumonitis from allantoic fluid, estimates have been made of numbers of elementary bodies, LD_{50} for chick embryos and of nitrogen, phosphorus and nucleic acid composition of virus suspensions. In order to meet the rigorous requirement of freedom from salt contamination in suspensions to be counted in the electron microscope, unphysi-

ologic suspending media have been used which jeopardize infectivity and, to an uncertain extent, may interfere with chemical integrity of the elementary bodies. Within the limits imposed by the requirements of electron microscopic counts it has been possible to derive minimum elementary body : chick embryo LD_{50} ratios between 360 and 1000. During repeated centrifugation and resuspension of virus particles in distilled water or ammonium acetate-albumin solutions this rat o reaches 10^6 with little apparent morphologic change in elementary bodies. N trogen ana yses correlated with counts have indicated that, in repeated washes in distilled water, limiting values of 2.1 to 4.6 $\times$ 10^{-15} gN/particle cannot be reduced without proportionate particle loss. Microspectrophotometric measurement of UV absorpt on, and pentose and desoxpentose color reactions following cold, then hot, perchloric acid extraction of elementary body suspensions, yielded values of 1.2–2.5 $\times$ 10^{-15}g DNA/particle without demonstrable RNA. Phosphorus content of such fractions has varied from 20.0-69.4% DNA, probably indicating insufficient P fractionation by the perchloric acid method as applied. From values for DNA, and for nitrogen $\times$ 10, the average particle mass was estimated at 2.5–5 $\times$ 10^{-14}g with 5–10% DNA.

Adaptation of influenza virus to mouse lungs. FRED M. DAVENPORT. *Dept. of Epidemiology and Virus Lab., Univ. of Michigan School of Public Health, Ann Arbor.*

Lines of a strain of Type B influenza virus have been established in mice by serial passage at 3 day intervals of unadapted virus in various dilutions. At appropriate passages, a comparison was made between these lines with respect to the presence of viral hemagglutinin, the yield of virus infectious for eggs, and the presence in the infected lungs of an inhibitor of viral hemagglutination. It was found that in the infected lungs the appearance of lesions correlated with the appearance of hemagglutinin and the disappearance of a substance which inhibits hemagglutination, while the concentration of virus as measured by infectivity for eggs was not regularly increased. Similar studies employing an A prime strain of influenza virus showed variations from this pattern. In addition it was shown that hemagglutination by the adapted lines of virus was inhibited by inhibitor in normal mouse lung to a lesser degree than was the unadapted line of the same strain. The rate at which virus became adapted to mouse lung was not markedly influenced by the dilution of virus used for passage.

Presence of Forssman antigen in inbred mice. ISRAEL DAVIDSOHN AND KURT STERN.* *Dept. of Pathology, Chicago Med. School, and Mount Sinai Med. Research Fndn., Chicago, Ill.*

The predominant part played by the mouse in cancer research makes it desirable to further investigate the controversial issue whether or not mouse tissues contain the Forssman antigen. Earlier affirmative statements in the literature were contradicted more recently. We reported previously the presence of natural antisheep agglutinins not of the Forssman type in 13 inbred mouse strains, with considerable variation of incidence and titer of antibodies depending on the strain. The question had to be investigated whether the differences in the occurrence of antibody may be paralleled by differences in the presence of Forssman antigen in the tissues. Immunization of rabbits with mouse tissues and absorption experiments with the immune serums demonstrated the presence of Forssman antigen in normal and neoplastic mouse tissues regardless of the strain of origin. In the present work the antigenic composition of cellular fractions of mouse and guinea pig tissues was studied. In particular, findings were compared between strain C57 Black mice with high titers of natural antisheep agglutinins and C3H mice without these antibodies. Microsome and mitochondrial fractions were found to have the highest concentration of Forssman antigen, again independent of strain derivation. The evidence so far collected speaks in favor of placing the mouse into the 'guinea pig group' of the Forssman system. (Supported, in part, by a research grant from the National Cancer Institute, National Institute of Health, U. S. Public Health Service.)

Antagonism between two successive aromatic precursors, shikimic acid and 5-dehydroshikimic acid. BERNARD D. DAVIS. *U. S. Public Health Service Tuberculosis Research Lab., Cornell Univ. Med. College, New York City.*

It has been shown hat in *Escherichia coli* 5-dehydroshikimic acid (DHS) and shikimic acid (SA) function as successive intermediates in the synthesis of tyrosine, phenylalanine, tryptophan, *p*-aminobenzoic acid, and *p*-hydroxybenzoic acid (POB). Mutants blocked before DHS require these 5 compounds, and can substitute DHS or SA for this requirement. Mutants blocked between DHS and SA, however, can respond to SA as a substitute for only the last 3 of these 5 metabolites. This limitation is due to the heavy accumulation by these strains of DHS, which competitively interferes with SA utilization. This competition was demonstrated in a double mutant in which the presence of blocks both before and after DHS allowed this compound to be tested as though it were an artificial inhibitor. In the course of these studies, striking preferential synthesis of the products of the common precursors could be shown: increasing DHS/SA ratios, from 0.2–30,

were required to interfere successively with synthesis of the above 5 aromatic metabolites, from tyrosine to POB. Evidence exists for similar antagonisms involving other intermediates in this sequence. Since in general successive metabolic intermediates have a close structural resemblance, competitive inhibition within a single biosynthetic chain may be quite widespread.

Determination of vitamin B_{12} by means of its combination with *L. leichmannii* resting cells. ROBERT L. DAVIS* AND BACON F. CHOW. *Dept. of Biochemistry, Johns Hopkins Univ. School of Hygiene and Public Health, Baltimore, Md.*

Both the growing and 'resting' cell of *L. leichmannii* (ATCC 4947) can incorporate vitamin B_{12} from the environment; the latter, in greater magnitude. These findings enabled us to develop some techniques useful in the rapid concentration and estimation of both radioactive and non-radioactive vitamin B_{12} present in a variety of biological fluids. This was accomplished by the addition of a suspension of 'resting' cells of *L. leichmannii* to given volumes of the test fluid. After standing at room temperature for approximately 30 minutes the microorganisms, which by then would have taken up quantitatively the vitamin B_{12}, were centrifuged and washed with isotonic saline solution. With samples containing radioactive vitamin B_{12}, the washed organisms were transferred to a planchet and the radioactivity measured. To estimate the non-radioactive vitamin B_{12}, 2 quantitative procedures were developed. *1*) The microbiological assay, in which the washed cells were suspended in normal saline solution and heated at 60° C. for 1 hour, and the resulting suspension was assayed microbiologically for vitamin B_{12}; *2*) an isotope-dilution method, in which use was made of a standard curve relating the uptake of radioactivity and the total quantity of vitamin B_{12} consisting of a constant amount of radioactive and increasing amounts of non-radioactive vitamin B_{12}. The uptake of vitamin B_{12} by *L. leichmannii* was effective over a wide range of pH's but was diminished at salt concentration above isotonicity. The advantages of our procedure lie in its rapidity, ease and comparative lack of inhibitors.

Effect of specific therapy on serologic and immunologic response in scrub typhus. FRED H. DIERCKS* AND JOSEPH E. SMADEL. *Army Med. Service Grad. School, Washington, D. C.*

OX-K agglutinins appeared early the second week of fever in 19 untreated cases of scrub typhus and reached a maximum mean titer of 1280 at the end of the third week; since the diagnosis here depended on positive Weil-Felix tests, all patients responded. Approximately 85% of the 13 patients with naturally acquired disease treated with PABA and of the 49 given specific antibiotics developed OX-K responses similar to the untreated patients with an average maximum titer of 640. None of the treated or untreated natural cases relapsed. Sixty-one volunteers infected by exposure in hyperendemic areas were given 24-hour courses of specific antibiotics during the first week of fever. Three-fourths of the 27 treated during the fourth to eighth day developed agglutinins at the usual time, average titer 640, while only $\frac{2}{3}$ of the 34 treated in the first 3 days did so. Agglutinins did not appear in the last group until the 13th day but reached 640 by the 21st day. Relapses occurred in $\frac{1}{3}$ and $\frac{2}{3}$, respectively, of the volunteers in the 2 groups. Specific therapy as usually employed in scrub typhus has little effect on the OX-K response. When given early, however, it may reduce and delay the appearance of agglutinins. It does not affect development of immunity which normally overcomes infection late in the second week. But if the therapeutic effect is lost before this time, relapses frequently occur.

Half-life of homologous gamma globulin (antibody) molecules in man, dog, rabbit, guinea pig and mouse. FRANK J. DIXON, DAVID TALMAGE* AND PAUL H. MAURER.* *Dept. of Pathology, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.*

The life of antibody molecules is a vital consideration in the study of the dynamics of immunity. In a species such as the rabbit where high serum antibody concentrations develop, this life has been measured by quantitative immunochemical determinations of passively administered homologous antibody. However, in other species such as man, dog, guinea pig and mouse, where lower antibody concentrations are the rule, this type of determination is extremely difficult. For such species it seems desirable to use I^{131} labelled homologous gamma globulin to measure the life of antibody molecules. I^{131} gamma globulin half-lives are in agreement with the published, immunologically determined half-lives of passively transferred homologous antibody. In the rabbit both methods yield half-life values of 4–5 days. In adult humans the half-life of I^{131} homologous gamma globulin (Lederle and Cutter) is 12–14 days. However, in infants preliminary observations show a longer half-life, indicating a difference in catabolism of gamma globulin between infants and adults. In dogs I^{131} canine gamma globulin (Armour) has a half-life of 7.6 days, which agrees well with the value obtained by others using C^{14} labelled amino acids as tracers. Values will be given for guinea pigs and mice. The life of homologous gamma globulin (antibody) molecules appears to be related to metabolic

characteristics of both the species and the age of the subject involved. (This study was supported by Atomic Energy Commission contract No. AT(30-1)-1205).

Evidence for propagation of virus of serum hepatitis in chick embryo. MILES E. DRAKE,* WERNER HENLE, GERTRUDE HENLE* AND JOSEPH STOKES, JR. *Children's Hosp. of Phaladelphia, and Univ. of Pennsylvania, Philadelphia.*

Evidence has been obtained which indicates that the virus of serum hepatitis (SH) can be carried through 15 passages in the amniotic cavity of the chick embryo. *1)* Intramuscular injection of amniotic fluid of the 9th and 15th passage into volunteers induced hepatitis without jaundice in 4 out of 6 and 2 out of 5 individuals, respectively, after incubation periods in excess of 60 days. *2)* Serum taken from 1 of the patients in the first group during the acute stage of illness, when given to additional 4 volunteers, resulted in 3 cases of jaundice, and one of hepatitis without jaundice. Challenge of the volunteers of the 1st group with natural virus (icterogenic serum) 8 months after the primary injection led to a second attack of hepatitis in 2 of the individuals. One of these was the donor of the serum referred to under *2*. Among the 5 controls 2 cases of jaundice and one of non-icteric hepatitis were recorded. Possible reasons for the absence of solid immunity after the first exposure will be discussed.

Correlation between protein combining capacity and ability of haptens to provoke delayed allergic skin responses. HERMAN N. EISEN, SIDNEY BELMAN* AND LEO ORRIS.* *Inst. of Industrial Medicine, New York Univ. Post-Grad. Med. School, New York City.*

A group of 8 2,4 dinitrophenyl compounds (D . . .) differing from each other in the substituent on the 1 carbon were studied in regard to ability to produce, on skin application, delayed allergic skin responses in guinea pigs and men who had been previously sensitized by 2,4 dinitrofluorobenzene (DF). Four of the compounds elicited skin lesions (elicitors), and 4 did not (non-elicitors). *In vitro*, elicitors readily combined irreversibly with protein (beef gamma globulin), particularly with lysine ϵ-NH_2; however, the non-elicitors did not so combine. Evidence for *in vivo* combination with skin protein was obtained by spraying skin of guinea pigs with each compound, removing the skin and subjecting it after acid hydrolysis to chromatographic analysis by Sanger's technique. N^5-D-lysine was demonstrated in hydrolyzates from animals treated with elicitors. Appropriate controls indicated that this derivative had been formed *in vivo*. After treatment with non-elicitors, no D-amino acids were recovered from hydrolyzates. Of the 4 elicitors, 1 (DSO_3) produced lesions in man but not in guinea pigs. The anomalous behavior of DSO_3, on account of its highly ionic character, may be attributable to feeble penetration of guinea pig skin. It is concluded that combination with skin (epidermal) protein through the formation of strong (covalent) bonds is a necessary condition for the production of delayed allergic skin lesions by haptens.

Alteration in pathogenicity for monkeys of Brunhilde strain of poliomyelitis virus following cultivation in human tissues. J. F. ENDERS, T. H. WELLER* AND F. C. ROBBINS.* *Research Div. of Infectious Diseases, Children's Med. Center, Boston, Mass.*

The propagation of poliomyelitis viruses in cultures of human tissues has made available a means of assay of viral infectivity that is independent of measurements carried out in living animals (*Proc. Soc. Exper. Biol. & Med.* 75: 370, 1950). Comparative titrations were done in roller tube cultures and in rhesus monkeys of the infectivity of the Brunhilde strain of virus maintained for various periods in suspended cell cultures of human embryonic skin-muscle. Virus of the second tissue culture passage caused paralysis in the monkey when diluted 10^{-3} and a cytopathogenic effect in roller cultures when diluted $10^{-5.2}$ (corrected to a volume of 1 ml., i.e. the size of inoculum used in the monkeys). Tenth passage virus caused paralysis in a dilution of 10^{-1} but still titrated $10^{-5.2}$ (correction as above) in tissue culture. In an attempt further to reduce the virulence of this material, 2 additional passages were made in roller cultures using as inocula minimal tissue-culture-infecting doses of virus. This procedure was adopted on the assumption that virus particles of low virulence for the animal might be segregated from those of higher virulence possibly present in smaller numbers. Undiluted fluid from the first roller culture passage induced paralysis in 1 of 6 monkeys. Its tissue culture titer was $10^{-5.2}$ (see above). Undiluted fluid of the second roller culture passage paralyzed 2 of 9 monkeys; its tissue culture titer was $10^{-5.8}$ (see above). Allowing for the difference in the volume of the inocula used in the 2 methods, it can be calculated that these monkeys received approximately 100,000 to 1,000,000 tissue-culture-infecting doses. These data indicate that the pathogenicity of a non-murine strain of poliomyelitis virus may be altered upon cultivation *in vitro*.

Persistence of antigen in mouse. JOHN O. ERICKSON, DOROTHY M. ARMEN* AND RAYMOND L. LIBBY. *Radioisotope Lab., Wadsworth General Hosp., Veterans Administration Center, and*

Dept. of Radiology, School of Med., Univ. of Calif., Los Angeles.

Tobacco mosaic virus (TMV), a high molecular weight nucleoprotein obtained from diseased tobacco plants, can be readily labeled with P^{32} and is highly antigenic in experimental animals. Its large size and characteristic rod-like shape permit its identification with the electron microscope. These facts suggest the use of this protein for immunological studies concerning the persistence of antigen. White mice were injected intravenously with either labeled or ordinary TMV and sacrificed in groups of 5–10 at appropriate time intervals. The pooled livers were homogenized and extracted with 0.1 M phosphate buffer at pH 7.6. Two types of experiments were carried out on these extracts to determine how long after injection TMV could be detected. *1*) This was tested by means of the specific precipitin reaction with added carrier TMV and rabbit anti-serum. It was found that labeled TMV could be detected in the extracts as long as 8 days after injection. *2*) Examination of concentrated extracts (uranium shadowed) from animals receiving non-labeled TMV in the electron microscope revealed the presence of particles strikingly similar to TMV in size and shape even after 14 days. It would appear from these studies that this antigen is not rapidly destroyed in the mouse.

A quantitative study of serological cross reactions of certain *Shigella flexneri* types. SAMUEL B. FORMAL* AND EDGAR E. BAKER. *Dept. of Microbiology, Boston Univ. School of Medicine, Boston, Mass.*

Homologous and heterologous antibodies in absorbed and unabsorbed sera prepared against *Shigella flexneri* Types IA, IB and III were determined by the quantitative agglutinin technic of Heidelberger and Kabat. Only the data obtained with *Sh. flexneri* Type IB antiserum will be presented at this time. An unabsorbed rabbit serum prepared with Type IB and tested with Types IA, IB and III contained 0.080, 0.199 and 0.072 mg. antibody N/ml. of serum respectively. Quantitative data will be presented to show that the Type IA cells will not remove all of the I antibody in a Type IB serum. Further, antibody directed against the 6-group antigen common to Types IB and III reacted almost quantitatively with both Type IB and III cells. The significance of these data will be discussed in terms of the antigenic structure of these microorganisms.

Infection of man with avirulent rickettsiae of epidemic typhus (Strain E). JOHN P. FOX, MARTHA EVERRITT AND PRAVIN N. BHATT (introduced by JOHN Y. SUGG). *Epidemiology Section, Dept. of Tropical Medicine and Public Health, Tulane Univ. School of Medicine, New Orleans, La.*

This report described efforts to confirm and extend observations, first made in Spain, as to the course and outcome of human infections with an 'avirulent' strain of *R. prowazeki* (Strain E). A total of 32 human volunteers were infected with graded doses of viable Strain E rickettsiae, from 10^6–10^2 egg-infective doses (EID), given by various routes (intramuscular, subcutaneous and intradermal). Clinical reactions of brief duration, which consisted chiefly of fever, headache and reaction at the site of inoculation, occurred within from 18–48 hours post-inoculation among most of those receiving the larger inocula (10^5 or 10^6 EID). Occasional second febrile episodes of uncertain significance occurred at from 10–14 days. Serologic and agent-isolation studies suggest that human infection did not occur when doses of 10^3 EID or less were inoculated. Rickettsemia was frequently but irregularly detected between 3 and 21 days post-inoculation. Complement-fixing antibodies against commercial epidemic typhus antigen appeared only in low titer and most regularly in persons inoculated with 10^5 or 10^6 EID by the intradermal and subcutaneous routes. Antibodies against a Strain E antigen were apparent in consistently higher titer and in the sera of nearly all persons receiving doses of 10^4 EID or more. That Strain E differs antigenically from unmodified *R. prowazeki* is a possibility which remains to be investigated.

Effect of vaccination and sulfonamide therapy on experimental toxoplasmosis. J. K. FRENKEL (introduced by DOUGLAS H. SPRUNT). *Rocky Mountain Lab., Hamilton, Mont.*

Mice were vaccinated subcutaneously with toxoplasma of lesser pathogenicity, and groups were superinfected daily with a highly pathogenic strain. Intracerebral or intraperitoneal challenge with about 5,000 LD_{50} 6–8 days after vaccination resulted in delayed death, and 10 days after vaccination in survival. Mice challenged subcutaneously 1 day after vaccination lived longer than the unvaccinated controls, and survived when challenged after the 5th day. In mice vaccinated only (and not superinfected), dye test antibodies appeared on the 7th day and reached a maximal titer of about 1:1000 on the 12th day, at which time complement-fixing antibodies made their appearance. When infected with a pathogenic strain and treated prophylactically with Na-sulfadiazine-merazine mixtures in the drinking water, ranging from an ED_0 (no drug) to an ED_{100} (100% effective dose—15 mg.%), mice showed a gradual increase in survival time concomitant with the increase in survival rate. Using an ED_{100}, prophylactically, intraperitoneally or sub-

cutaneously infected mice survived if treated for the first 2–6 days after infection. There was no gradual increase in the survival period such as was observed after vaccination and challenge, or after chemotherapy with less than an ED_{100}. Similar dye test titers ranging from 1:256–1:2048 were present in the mice dying and those surviving with treatment. Mice infected with certain strains of toxoplasma died days to weeks after cessation of chemotherapy of a month's duration. Inocula gave lower titers in mice receiving sulfonamides than in untreated mice.

Folin-Wu preparation of blood group specific substance A. EARL B. GERHEIM, GEORGE R. LOGWOOD* AND HORACE M. FLOYD.* *Div. of Research, Univ. of Detroit School of Dentistry, Detroit, Mich.*

A comparative study was made of the blood group specific substance A products prepared by the Folin-Wu technic previously described (*Proc. Soc. Exper. Biol. & Med.* 72: 394–395, 1949), using Difco neopeptone and 1:3000 pepsin prepared from hog stomach. The titer was determined by the inhibition of isoagglutination. A serial dilution was made of the material dissolved in physiologic saline, starting with 0.1%. The titers are reported in the usual manner. Eight isoagglutinin units were used in all tubes. Neopeptone gave a product free of sulfate and tungstate that habitually had a titer of 512. Pepsin on the other hand yielded a product with a titer as high as 16,384, but difficulty was encountered in dialysing away the tungstate. It was found that no amount of dialysis would remove the tungstate unless the pH was first adjusted well on the alkaline side (pH 10 was usually used). Nitrogen (micro-kjeldahl) and carbohydrate (orcinol method) determinations were made on the products prepared from both sources. The range of values follow:

SUBSTRATE	N %	CHO %
Neopeptone.....	4.83 ± 0.40	11.58 ± 1.26
Pepsin.....	9.85 ± 0.59	14.85 ± 6.05

Leptospiral etiology of Fort Bragg fever. W. S. GOCHENOUR, JR., J. E. SMADEL, E. B. JACKSON, L. B. EVANS* AND R. H. YAGER. *Army Med. Service Grad. School, Washington, D. C.*

Tatlock (*J. Clin. Investigation* 26: 287, 1947) recovered an agent transmissible in embryonated eggs and laboratory animals from a patient with Fort Bragg Fever in 1944. Using 104th passage material, he reproduced Fort Bragg Fever in volunteers in 1946. The agent has since been maintained here and at Yale University by serial transfer in eggs and hamsters. The possibility that the agent might be a leptospira was examined several times, but negative results were obtained with the methods then employed. Agglutination-lysis tests on paired sera from the 1946 volunteers using a wide variety of leptospirae showed that these persons developed high antibody titers against *L. autumnalis*. With this lead, cultural techniques yielded a leptospira from material of the 259th passage of the Fort Bragg agent. Tatlock's original patients, whose sera were on file, demonstrated a rise in antibody titer against this leptospira, as did 11 of 22 other patients with clinical Fort Bragg Fever during 1943 and 1944. Chimpanzees experimentally infected in New Haven with the Fort Bragg agent (*Proc. Soc. Exper. Biol. & Med.* 67: 263, 1948) likewise developed such an antibody response. The evidence clearly indicates that the agent which has been maintained under the name 'virus of Fort Bragg Fever' is a leptospira. Definitive identification of this leptospira, previously unreported in the United States, is in progress.

Complement-fixing antibodies to Type 2 poliomyelitis virus in a normal population. NATHAN GOLDBLUM* AND JOSEPH L. MELNICK. *Section of Preventive Medicine, Yale Univ., New Haven, Conn.*

Following the adaptation of the MEF1 strain of poliomyelitis virus to infant mice, Casals and Olitsky were able to prepare an antigen for the detection of complement-fixing (c-f) antibodies to Type 2 poliomyelitis virus. Using this antigen, we have followed the rise and fall of c-f antibodies in a population apparently heavily exposed to poliomyelitis virus. Sera collected from normal persons in the vicinity of Cairo, Egypt, in 1950, had been previously studied in this laboratory for neutralizing antibodies to the 3 types of poliomyelitis virus. Neutralizing antibodies were acquired relatively early in life and this was particularly true for Type 2 (Lansing) which were present in more than 90% of the sampled population over 3 years of age. For Type 2 virus, the age distribution of c-f antibodies did not parallel that of neutralizing antibodies. Both antibodies rose together during the first year of life, but the incidence of c-f antibodies was never over 50% in any age group. Beyond the age of 5–9 years, the incidence of c-f antibodies dropped very close to the zero level, in contrast to the neutralizing antibodies which were maintained in the adult population. These data suggest that the lifespan of c-f antibodies to poliomyelitis virus is of shorter duration than that of neutralizing antibodies. C-f antibodies appear to be an expression of relatively recent infection with poliomyelitis virus, in contrast to neutralizing antibodies which indicate either recent infection or one long past.

A bacterial enzyme which inactivates thromboplastic suspensions. S. GOLLUB, D. FELDMAN, F. E. KAPLAN, D. C. SCHECHTER AND D. R.

MERANZE (introduced by L. H. TUFT). *Research Dept., Mt. Sinai Hosp., Philadelphia, Pa.*

It was previously reported from this laboratory that a bacterial contaminant caused a sudden potency loss of tissue thromboplastic suspensions. Further work has shown the causitive organism, previously identified as Bacillus subtilus, to be a xylose positive strain of Bacillus cereus. A cell-free enzyme preparation has been obtained by growing the organism on a medium of brain heart infusion. A simple medium containing only salts and sugar supported growth; however, no enzyme was elaborated. The enzyme is apparently adaptive in nature. It appears to be relatively heat-stable. Crude preparations remained active after 1 hour at 75° C. and frequently after 5 minutes at 100° C. Crude enzyme preparations have been highly active against all forms of tissue thromboplastin thus far investigated, including rabbit brain, rabbit lung, beef lung, beef brain, human placenta and several commercial preparations. Preliminary experiments aimed at determining the mode of action of this enzyme, named 'thromboplastinase' for identification purposes, have indicated no protease, lecithinase, lipase, phosphomonoesterase, RNAase and DNAase activity. An apparently quantitative split of phosphate relative to inactivation of thromboplastin has been found for one thromboplastin substrate, human placenta. Copper and tin inhibited enzyme activity.

Toxins of *Pasteurella pestis*. K. GOODNER, P. BARTELL AND L. PANNELL. *Jefferson Med. College, Philadelphia, Pa.*

Toxic derivatives of *P. pestis* have been described by many investigators. The technical procedures used have differed widely. Repetition with these procedures has given some evidence that in most instances the end-products have represented mixtures of toxic principles in proportions as determined by the conditions employed. A highly toxic material has been derived from this organism by treating suspensions with a solution of sodium desoxycholate. It would appear that the potency of this last preparation can be increased by the presence of molecular oxygen.

Synergistic effect of Roentgen radiation and cortisone upon pathogenicity of Coxsackie virus. ASA B. GRAHAM,* ALVAR A. WERDER,* JEROME T. SYVERTON AND JACK FRIEDMAN. *Depts. of Bacteriology and Immunology and of Radiology, Univ. of Minnesota, and Mt. Sinai Hosp., Minneapolis.*

It has been learned in this laboratory that Roentgen radiation and cortisone enhance remarkably the pathogenicity of infectious agents. The present report deals with the effects *1*) of Roentgen radiation, *2*) of cortisone acetate (Cortone, Merck) and *3*) of these 2 agents in combination upon the pathogenicity of Coxsackie virus for adult Swiss albino mice. The Minnesota-1 strain, type 4, group A, Coxsachie virus was employed. This strain regularly paralyzes and kills suckling mice but it is avirulent for adult mice. Graded single doses of cortisone, 0.1–9.0 mg., and whole body Roentgen irradiation from 50–500 r, when given within 24 hours before the injection of virus, were found for adult mice to result in an enhancement of pathogenicity as shown by weight loss, histologic changes, and death. The radiation factors were 60 cm. target-skin distance; 250 kv.; 15 ma.; filter 0.5 mm. copper, 1 mm. aluminum; HVL 1.35 mm. Cu; size of field 14.0 cm. in diameter. The output is, as determined by a Victoreen r-meter, 41.5 r/min. in air. The findings for a single experiment will serve to illustrate the effects. Adult mice which had been prepared *1*) by a whole body irradiation, 150 r, survived (11/11) injection of virus; *2*) by cortisone, 2.0 mg., 6 of 18 died; *3*) x-ray (150 r), and cortisone (2 mg.) in combination died (11/11) of virus infection. The results that were obtained for the 1156 adult Swiss albino mice employed as test recipient hosts in this study are presented summarily.

Transfer of lymph node cells from rabbits injected with antigens. T. N. HARRIS, SUSANNA HARRIS* AND MIRIAM B. FARBER.* *Children's Hosp. of Philadelphia, and Univ. of Pennsylvania, Philadelphia.*

Following the demonstration by Chase of the transfer of sensitivity to picryl chloride by means of cells teased from lymph nodes and spleens (CHASE, M. W. *Federation Proc.* 10: 404, 1951), it was observed that after the injection of dysentery bacilli into the feet of rabbits cells could be teased from the popliteal lymph nodes of these donor rabbits which, on injection into fresh rabbits, gave rise to the appearance of agglutinins in the serum of the recipient animals. (HARRIS, S. AND T. N. HARRIS. *Federation Proc.* 10: 409, 1951). In further studies the interval of time has been varied between the injection of antigen into the donor and removal of its lymph node cells. It has been found that if this interval is 3 or 4 days, antibodies appear in the recipient on the first day following the transfer of lymph node cells. If the interval is reduced to 2 days, antibody appears in the recipient on the second day after transfer, but the titers then follow the pattern typical of these experiments. If only 1 day is allowed between injection and cell-collection in the donor, the recipient's serum shows antibody beginning the third day after transfer. The relative roles in this effect associated with a function of viable cells and active immunization, respec-

tively, were studied by x-irradiation of the recipient rabbits and/or the transferred cells. In other studies the influence of such agents as cortisone and enzyme poisons on the transfer effect were investigated.

Serological differences among native and clinical dextrans. EDWARD J. HEHRE* AND JAMES M. NEILL. *Dept. of Bacteriology and Immunology, Cornell Univ. Med. College, New York City.*

All the native and clinical dextrans that we have tested possess serological reactivity, and can be separated into 2 broad groups on the basis of their cross-reactions with standardized antipneumococcus rabbit serums. *Group A*: precipitate with type 12 as well as with types 2 and 20 antiserums. *Group B*: precipitate only weakly or not at all with type 12, but precipitate well with types 2 and 20 antiserums. Marked differences have been found among the dextrans of each of these groups, in respect to capacity to fix complement with typhoid rabbit antiserums. In addition, there are prominent quantitative serological differences between native and clinical dextrans, which are related to molecular size. However, the qualitative serological features (demonstrable with pneumococcus and with typhoid antiserums) that serve to distinguish one native dextran from another are not lost during the partial hydrolysis involved in the manufacture of the clinical products. Among the clinical dextrans used as plasma volume extenders, the products from one manufacturer had the properties described for Group *A*; the products from 2 other manufacturers had the properties of Group *B*, but differed greatly from each other in capacity to fix complement with typhoid rabbit antiserums. The same features that differentiated these partially hydrolyzed products were also possessed by the native dextrans from the 3 manufacturers. Studies toward correlating the serological differences among dextrans with differences in their chemical structure are being made in cooperation with the Northern Regional Research Laboratory of the Department of Agriculture. (Supported by grant, National Heart Institute, U. S. Public Health Service.)

Antigenic analysis of influenza viruses by immune serum neutralization in ovo. MAURICE R. HILLEMAN AND FRANK L. HORSFALL, JR. *Hosp. of Rockefeller Inst. for Med. Research, New York City.*

The antigenic relationships of 15 selected strains of influenza A virus were studied by immune serum neutralization *in ovo* using chicken antisera. The results were correlated with those obtained earlier on the same strains by the hemagglutination-inhibition technique. Five strains of so-called A prime virus, recovered in successive years from 1947–1951, showed a progressive change in antigenic composition similar to that demonstrated by the hemagglutination-inhibition procedure. Eight strains of virus, recovered in widely different parts of the world during 1950–51 and shown to be markedly homogeneous on analysis by hemagglutination-inhibition, were somewhat less homogeneous on serum neutralization *in ovo*. In general, the results of the neutralization experiments corresponded closely with and provided support for the results of antigenic analysis by hemagglutination-inhibition. However, the neutralization technique almost always showed somewhat more marked antigenic differences than did the hemagglutination-inhibition procedure. An analysis of the degree of correlation between and the relative merits of the *in vitro* and *in vivo* procedures will be presented.

Poliomyelitis virus in the blood of orally infected monkeys and chimpanzees. DOROTHY M. HORSTMANN. *Section of Preventive Medicine, Yale Univ., New Haven, Conn.*

Viremia is considered to be of rare occurrence in human poliomyelitis, since virus has been isolated from the blood of a patient only once, in spite of many trials. One explanation for the negative results is that perhaps viremia usually occurs in the incubation period and by the time clinical symptoms appear, it is too late to demonstrate it. This possibilty prompted the present series of experiments, in which cynomolgous monkeys and chimpanzees were fed 5 ml. 20% suspension of the Y-SK strain of poliomyelitis virus and, beginning 24 hours later, were bled daily for 5–7 days. The bloods from the chimpanzees and from the monkeys which subsequently developed paralysis were then tested individually by intracerebral inoculation into rhesus monkeys. Two of 5 cynomolgous monkeys proved to have virus in the blood 4 and 5 days after feeding, which was 6 and 7 days before the onset of paralysis. As is usual following oral infection, the chimpanzees remained well, but 2 of 4 were shown to have viremia on the 4th and 5th days after feeding. In view of these findings, the possibility exists that transient viremia occurs during the incubation period in the natural disease.

Duration of immunity in chimpanzees vaccinated with formalin inactivated poliomyelitis virus. HOWARD A. HOWE. *Johns Hopkins Univ., Baltimore, Md.*

During a study of immunity to poliomyelitis in chimpanzees it has been possible to make some observations on the duration of immunity in animals vaccinated and subsequently challenged by oral inoculation of homologous virus. The group consisted of 9 animals (F 605–613) which were immunized over a period of 3 months with a 10% suspension of rhesus monkey cord contain-

ing formalin inactivated Brunhilde virus. The preparation was judged to be incapable of initiating infection because of its failure to produce paralysis or lesions when 2 cc. were inoculated intracerebrally into each of 10 rhesus monkeys. (This method is capable of demonstrating Brunhilde virus in dilutions of 10^{-7}.) Each of the 9 chimpanzees received a total of 6 gm. of cord after which they were challenged by feeding 5 cc. 20% Brunhilde III B-501 (about 300,000 ID_{50} doses). Following this challenge all of the animals remained free of paralysis although 4 shed virus in their stools for a brief interval. The animals remained in the laboratory for 7 months but were permitted no direct intercommunication. They were then sent to a custodial institution where they were aggregated in a single cage for 1 year. Finally after 19 months, 8 animals received a second identical challenge. Again no paralysis ensued. One animal which had not been infected after the first challenge became a transient virus carrier. It thus appears that the immunity induced by formalized virus is capable of enduring effectively for a considerable time. The possibility of a booster reinfection during the waiting period seems unlikely but does not essentially change the argument.

Neutralization of herpes simplex virus by human sera. ERNEST JAWETZ. *Div. of Microbiology and Medicine, Univ. of California School of Medicine, San Francisco.*

Most individuals acquire infection with herpes simplex virus in childhood and subsequently have specific antibodies in their serum. According to Burnet the character of these antibodies is 'all or none,' i.e. there is either a consistently high titer, or a complete absence of neutralizing antibodies. Such findings would be quite unique in the realm of infectious diseases and one wonders whether the technique employed (reduction in pock count by undiluted serum) might be responsible. A quantitative neutralization test was developed in embyonated eggs using an egg-adapted strain of herpes virus. Virus-serum mixtures (0.5 ml.) were inoculated into the yolk sac of 7-day old eggs and death was taken as endpoint. Several variables in the technique were explored. With both constant virus-serum dilution and constant serum-virus dilution methods, the preliminary incubation of virus-serum mixtures was of greatest importance. Using a virus with an LD_{50} of $10^{-5.2}$ the sera from 7 patients with recurrent herpetic lesions (diluted 1:30) neutralized 0.5–1.3 log virus when virus-serum mixtures were inoculated without incubation. The same sera neutralized 1.0–3.7 log virus after incubation at 37° C. for 1 hour. Another random series of sera (diluted 1:30) from persons with positive herpes skin tests neutralized from 0.3–3.7 log virus, and covered the complete range between these extremes. Thus there is no evidence of an 'all or none' character of anti-herpetic antibody in sera examined by this technique. When sera obtained from the same patient at different times were compared without incubation of serum-virus mixtures, titers appeared uniformly constant. However, when a constant serum-virus dilution technique with preliminary incubation was employed unequivocal fluctuations of amounts of virus neutralized were found in certain sera.

Effect of irradiaton on universal serologic reaction in rabbits. REUBEN L. KAHN AND HELENE V. PETRENCO.* *Serology Lab., Univ. of Michigan Hosp., Ann Arbor.*

Studies were carried out to determine whether irradiation of rabbits with x-rays affects their universal reactions. Eleven rabbits were examined for the normal precipitation levels of their universal reactions. Then 3 rabbits were given 200 r total body irradiation 3 times at weekly intervals; 3 rabbits 300 r under the same conditions; 3 rabbits 400 r under the same conditions; 2 rabbits served as controls, receiving no irradiation. Universal reactions were then performed 14, 45 and 70 days, respectively, after the third irradiation of the experimental animals, and simultaneously, reactions were also performed of the control animals. It was found that the precipitation level of the universal reaction following irradiation was increased in the irradiated rabbits. One of the animals which received 200 r 3 times showed but minimal increase in precipitation. The most marked increase in precipitation was shown by the rabbits which received 300 r 3 times. It is possible that 400 r given 3 times interfered to some extent with the increase in precipitation. The 2 control rabbits showed no change in their universal reactions. The results indicate that under the experimental conditions herewith presented, irradiation increases lipid antigen-antibody reactivity. Earlier irradiation experiments gave inconstant results; due, it is believed, to the fact that 6–7 cc. of blood for universal reactions were repeatedly withdrawn every 2 or 3 weeks. The frequency of these bleedings may have depressed precipitation in the universal reaction.

Effect of cortisone on nucleoproteins of lymph nodes. EDWARD H. KASS AND MARY I. KENDRICK (introduced by MAXWELL FINLAND). *Thorndike Memorial Lab. and Harvard Med. School, Boston, Mass.*

Some of the cellular mechanisms by which cortisone depresses resistance to infection were investigated. Cortisone was administered to rabbits of standard weight in doses of 10 mg. daily and the effects on the popliteal lymph nodes ob-

served. The mean weights of the nodes decreased rapidly to about 35% of the normal weight as a consequence of daily administration of cortisone, and the concentration of desoxypentose nucleic acids (DNA) did not change significantly despite marked lympholysis. The concentration of pentose nucleic acids (PNA), however, decreased by about ⅓, and there were accompanying indications, histochemically and histologically, of decreased numbers of immature lymphoid cells containing PNA, with accumulation of macrophages. After the administration of cortisone for 5 days, as much as 7 days were required for the concentration of PNA and the size of the lymph nodes to return to their normal values. Rabbits were given homologous erythrocytes into one paw and foreign erythrocytes into the contralateral paw. The presence of foreign cells normally causes the regional nodes to undergo marked hyperplasia, with associated increases in concentrations of PNA. The lymph nodes in cortisone-treated animals were smaller than in controls, showed less hyperplasia in response to antigenic stimulation, tended to retain the erythrocytes longer in the nodes, and contained lower ratios of PNA to DNA than similarly treated nodes taken from animals that had not received cortisone. The data suggest that cortisone affects the PNA metabolism of lymph nodes of rabbits and that this interference may explain the lack of formation of antibody and perhaps the inhibition of hyperplastic responses in the antigenically-stimulated lymph node.

Passive immunity to vaccinia in newborns: Placental transmission of antibody; response to vaccinations. C. HENRY KEMPE, A. S. BENENSON AND J. R. JACKSON. *Div. of Pediatrics, Univ. of California School of Medicine, and George Williams Hooper Fndn., San Francisco.*

Vaccinia hemagglutination-inhibition titers (BENENSON, A. S., AND C. HENRY KEMPE. *Federation Proc.* 10: 402, 1951) of 182 mothers and their newborn infants were compared. Seventy-one infants had titers significantly higher than their own mothers', while only 5 were significantly lower. Mothers vaccinated within the past 5 years have higher titers than those vaccinated earlier. Their infants have significantly higher titers than infants of mothers vaccinated more than 5 years ago. Complement fixation tests of inactivated sera of mothers and their infants confirmed the presence of a specific antibody present in increased amounts in the infants. Two newborn infants with low titers were successfully vaccinated at 5 days of age and demonstrated a rise in antibody titer. Two newborn infants with very high antibody titers in their sera could not be successfully vaccinated despite careful technique and highly active inoculum. This finding probably represents instances of true 'no takes' based on passive immunity transplacentally acquired. Revaccination of these infants at 6 months of age resulted in a skin reaction maximal on the 5th and 6th day respectively, in the absence of an earlier sensitivity type reaction, suggestive of previous antigenic experience. This suggests that the response observed was an accelerated primary take or 'modified vaccinia' based on transplacental passive immunity. Serologic data on these patients will be presented.

Studies in complement-fixation. III. Influence of normal serum constituents in reaction with syphilitic serum and cardiolipin antigen. JOHN F. KENT. *Dept. of Serology, Army Med. Service Grad. School, Washington, D. C.*

Decreasing concentrations of syphilitic serum, prepared by diluting in normal serum or in buffered salt solution, were tested each with multiple concentrations of cardiolipin in the presence of a constant quantity of complement (C′). The tests were incubated under conditions (16–18 hours at 3–6° C.) ordinarily considered optimal for C′ fixation; sensitized erythrocytes were then added, and a period of 30 minutes at 37° C. was allowed for hemolysis. Percentage of hemolysis was measured by a precise spectrophotometric technic. The choice of diluent for the syphilitic serum influenced not only the degree of C′ fixation but also the optimal proportion of cardiolipin antigen to syphilitic serum. Thus, the use of normal serum as diluent resulted in partial inhibition of the capacity to fix C′, and considerably increased the concentration of antigen required for maximal fixation. Both effects appeared associated with the concentration of normal serum constituents. They were most prominent when the syphilitic serum was of high titer and there was greatest disparity in the amount of normal components represented by dilutions prepared in normal serum and in salt solution. A similar comparative study was conducted with syphilitic cerebrospinal fluid (CSF) diluted in normal CSF and in salt solution. The findings are discussed from the point of view of their importance in the standardization of antigen for diagnostic complement-fixation tests.

Localizing properties of anti-tissue antibodies purified with fractions of cell nuclei. LEONHARD KORNGOLD* AND DAVID PRESSMAN. *Sloan-Kettering Inst., New York City.*

The *in vitro* purification of anti-rat-lung sera was carried out with nucleohistone preparations obtained from rat liver, lung and kidney. The eluted antibody had the following localizing properties: *a*) Highest kidney localization was obtained with material eluted from kidney nucleohistone. *b*) Highest lung localization was obtained

with material eluted from lung nucleohistone. *c*) Highest liver localization, however, was with material eluted from lung nucleohistone, while the material eluted from liver nucleohistone showed very low localization in the liver. When the nucleohistones were fractionated further into histone, DNA and residual chromosome fractions, the residual chromosome fraction gave the best purification of the localizing antibodies. Purification of the anti-lung serum with residual chromosome preparations of liver, kidney and lung was also carried out and the results were similar to those obtained with the nucleohistones. Moreover, similar results were obtained with anti-rat liver serum purified with residual chromosomes with the exception that no lung localizing activity was isolated from any of the residual chromosome preparations. The presence of antigens in the nuclei capable of combining *in vitro* with localizing antibodies does not necessarily mean that localization *in vivo* takes place on these structures.

Immunization of humans with purified Vi antigen derived from *E. coli* 5396/38. MAURICE LANDY AND ESTHER LAMB (introduced by GEOFFREY EDSALL). *Immunology Div., Army Med. Service Grad. School, Army Med. Center, Washington, D.C.*

Vi antigen has been isolated in these laboratories, (*Federation Proc.* 10: 413, 1950) from *E. coli* 5396/38 by an ethanol-salt fractionation procedure and shown to be a polymeric acid containing nitrogen and acetyl groups but not in the form of N-acetyl hexosamine. Immunological studies revealed that the purified antigen possessed good immunogenic activity for mice, rabbits and humans. In the present study 300 subjects were given a single subcutaneous injection of 40 μg. of the purified Vi antigen. Blood samples were drawn immediately prior to and 21 days following injection of the antigen. Sera from all volunteers were titrated for Vi antibody by the hemagglutination technique (agglutination of erythrocytes sensitized with purified Vi antigen) while sera from approximately one-sixth of these individuals were available in quantities sufficient for analysis by the quantitative precipitin method. Vi hemagglutinin titers were found to be correlated with antibody nitrogen content, as determined on a weight basis ($P < 0.001$). Ninety-six per cent of the subjects developed antibody reactive with Vi sensitized erythrocytes. Information of a preliminary nature also was obtained indicating that Vi antibody was detectable 7 days following injection of antigen, reached maximum titer in 14–21 days, persisted for at least 18 months, was produced by an immunizing dose of as little as 10 μg. of antigen and possessed mouse protective potency against challenge with *S. typhosa*.

Kinetics of influenza virus hemagglutination-inhibition reactions. FRANK LANNI AND YVONNE THÉRY LANNI.* *Dept. of Bacteriology, Univ. of Illinois, Urbana, Ill.*

Hemagglutination-inhibition data obtained with mixtures of egg-white inhibitor (I), heated swine influenza virus (V), and chicken erythrocytes (R) have been interpreted by postulation of the following set of effectively irreversible bimolecular reactions: *1*) $V + R \xrightarrow{k_1} VR$; *2*) $V + I \xrightarrow{k_3} VI$; *3*) $VR + R \xrightarrow{k_5} VR_2$; and *4*) $VR + I \xrightarrow{k_7} IVR$. The ratio k_5/k_7 is assumed equal to k_1/k_3, and reaction between IVR and R is excluded. In this model, a virus particle contributes to hemagglutination only through formation of VR_2, which is prevented by reactions *2* and *4*. Combination with a *single* inhibitor molecule thus suffices for inhibition. When I and V react for a preliminary period in absence of R, the additional reaction $VI + I \rightarrow VI_2$ appears to contribute significantly through its effect in depleting I. Kinetic analysis yields for k_1 and k_3 the values 1.1×10^{-8} and 0.3×10^{10} cm^3 min^{-1}, respectively, all concentrations being expressed as particles (molecules) per cc. Comparison with the corresponding maximal theoretical values 1.2×10^{-8} and 2×10^{-10} (estimated for 100% collision efficiency) suggests that the interactions are predominantly ionic. This conclusion, reached previously for bacteriophage (see PUCK, GAREN AND CLINE, *J. Exper. Med.* 93: 65, 1951) accords with available information on influenza viruses, but requires more direct confirmation. A byproduct of the analysis is the estimated value 8×10^6 for the molecular weight of I. A minimal value 7.6×10^6 (subject to revision) was calculated previously from purely physical information (*J. Biol. Chem.* 179: 1275, 1949).

Poliomyelitis virus and antibody studies in roller tube cultures of monkey testicular tissue. NADA LEDINKO,* JOHN T. RIORDAN* AND JOSEPH L. MELNICK. *Sec. of Preventive Medicine, Yale Univ., New Haven, Conn.*

The cytopathogenic changes which, for most strains, parallel the multiplication of poliomyelitis virus in roller tube cultures of monkey testicular tissue may be specifically neutralized by immune sera, permitting an *in vitro* test for poliomyelitis antibodies. As immunologically related strains may differ in the degree of their cytopathogenic effect, strains known to produce marked fibroblastic degeneration should be used as representatives for each of the 3 virus types. Renewal of specific antibody each time the fluid bathing the tissue culture is renewed completely prevents virus induced fibroblastic degeneration, in contrast to the delayed degeneration ('virus break-through') evident when antibody is not

subsequently renewed. A linear logarithmic relationship exists between the quantity of virus used and the quantity of serum required to neutralize the virus in tissue culture. Using the cytopathogenic effect of poliomyelitis virus on fibroblasts proliferating in roller tube cultures of monkey testicular tissue as an indicator, 26 strains of virus were isolated from 40 human stool samples and from 2 human spinal cord samples. Identification of 12 of these strains as Type 1 (Brunhilde) was done by neutralization of this cytopathogenic effect with type specific antisera. Strains of virus having a weak cytopathogenic effect can be successfully typed in tissue cultures by immunizing monkeys with such strains and subsequently typing their sera in tests employing strong cytopathogenic strains belonging to each of the 3 known types of poliomyelitis virus. The methods described provide a relatively rapid and economical means of isolating and identifying strains of poliomyelitis virus.

Immunological and hormonal studies on the nature of genetic resistance to tuberculosis. MAX B. LURIE, SAMUEL ABRAMSON,* A. G. HEPPLESTON* AND ARTHUR M. DANNENBERG, JR.* *Henry Phipps Inst., Univ. of Pennsylvania, Philadelphia.*

There is evidence that each virulent bovine type tubercle bacillus that penetrates into the alveoli of natively resistant or susceptible rabbits generates a gross pulmonary tubercle; only a fraction of similarly localized human type tubercle bacilli generates a pulmonary focus. The number of tubercles thus generated by a given number of human bacilli is a function of native resistance; the greater the resistance, the fewer the tubercles. Human type bacilli in the lung of susceptible rabbits grow for a considerable time and are not destroyed for months after the infection. Such bacilli inhaled by natively resistant rabbits are destroyed within a few weeks. Resistant rabbits develop allergic sensitivity and antibodies more rapidly than susceptible animals. Variations in genetic resistance are essentially variations in the rate of development of acquired resistance. The number of human type tubercle bacilli necessary to generate a single tubercle in rabbits was greatly reduced by cortisone; conversely, this number was greatly increased by BCG vaccination. Cortisone interferes with the destruction of tubercle bacilli within the cells. Vaccination increases the intracellular destruction of bacilli. It is suggested that native resistance to pulmonary tuberculosis is controlled by the prevalence of alveolar phagocytes capable of acquiring an effective capacity to destroy tubercle bacilli. BCG immunization increases the prevalence of such cells while an excess of cortisone diminishes it.

Production of an altered pantothenate synthesizing enzyme as a result of mutation. WERNER K. MAAS (introduced by BERNARD D. DAVIS). *U. S. Public Health Service, Tuberculosis Research Lab., Cornell Univ. Med. College, New York City.*

Growth experiments have suggested that mutations blocking a biosynthetic reaction do so by preventing production of the corresponding enzyme. This hypothesis could be tested directly by enzyme studies on extracts of *Escherichia coli* mutants blocked in the synthesis of pantothenate from β-alanine and pantoic acid. Two mutants were compared with wild type: I requires pantothenate at any temperature; II, a temperature-sensitive one, requires it only above 30° C. Extracts of fresh or acetone dried cells of wild type yielded an active enzyme, stable up to 45°. Extracts of I were completely inactive. Extracts of II were active, but extraordinarily heat-labile, being rapidly inactivated at 25°. They could be protected by agents known to prevent protein denaturation such as sugars or neutral salts. In mixtures, extracts of wild type and II did not affect each other's stability. Similarly, extracts of I did not inhibit the wild type enzyme, nor did wild type extracts activate an enzyme from I. These mutations have therefore been shown to block a reaction by affecting the corresponding enzyme, rather than the environment. Furthermore, this effect involves, at least in the temperature-sensitive mutant, a qualitative change in the enzyme molecule.

Action of ACTH and cortisone on anaphylactic shock in the guinea pig. STANLEY MARCUS, JOHN H. CARLQUIST,* DAVID M. DONALDSON* AND GERALD M. CHRISTENSON.* *Dept. of Bacteriology, Univ. of Utah College of Medicine, and Latter Day Saints Hosp., Salt Lake City.*

Almost all literature on the subject indicates that ACTH and cortisone have little or no protective effect on anaphylaxis in the guinea pig. In all instances however large shocking doses, *i.e.* multiple ED_{50}s of antigen, have been used. The more precise experimental design which takes advantage of the dose-response relationship in anaphylaxis has been utilized to test the anti-anaphylactic effects of the aforementioned drugs. Rabbit anti-chicken egg albumin serum (Ab) was prepared in the usual manner and the Ab nitrogen content determined. Guinea pigs were passively sensitized by intracardial injection of Ab; 24 to 26 hours later the animals were challenged intracardially with 1.0 mg. of (Armour's) crystalline egg albumin. All animals were autopsied following death or 10 minutes after challenge. The ED_{50} dose was calculated by probit technque to be 12.4 $\pm$ 0.83 μg. Ab nitrogen. Neo-Antergan

protected guinea pigs against 6.5 ED_{50}s. None of 4 animals pretreated with Neo-Antergan showed any symptoms following challenge or at autopsy. Various amounts of ACTH (ACTHAR gel, Armour) and cortisone (cortisone acetate, Merck) did not protect animals against 1 ED_{80} (1.3 ED_{50}) of Ab. Other animals were sensitized with 1 ED_{80} of Ab and divided into groups. One group was untreated (control). Each animal in the second group was injected with a total of 40 USP units of ACTH preceding challenge and each animal in a third group was injected with a total of 80 mg of cortisone acetate preceding challenge. Following challenge all animals were autopsied; animals which died were autopsied immediately following the cessation of respiration. Animals which lived were killed by a blow to the head 10 minutes following challenge and autopsied. In all cases, lungs were quickly removed fixed in formalin and sectioned. The hormone treated animals which survived acute shock showed little or no peribronchial edema. A working hypothesis, based on the foregoing data, will be offered to explain the efficaciousness of steroid hormone therapy in the asthmatic state.

Quantitative immunochemical studies on the inhibition of urease by rabbit anti-urease. A. A. Marucci* and M. M. Mayer. *Dept. of Bacteriology, Johns Hopkins School of Hygiene and Public Health, Baltimore, Md.*

Quantitative precipitin analyses of the reaction between crystalline urease and rabbit anti-urease have yielded results essentially similar to those obtained with other protein antigens. Anti-urease precipitates urease completely in the region of antibody excess as well as in the zone of equivalence; thus, supernatant fluids at various points in these regions of the precipitin reaction contain no urease detectable by enzymatic measurement. However, the precipitated antigen-antibody complexes still possess enzymatic activity which ranges from 20–70% of that exhibited by urease alone. The degree of inhibition depends on the ratio of antibody to urease; with a large excess of antibody enzymatic activity is inhibited about 80%, while in the equivalence zone inhibition is about 30%. The inhibition is not primarily due to aggregation, but appears to be the result of steric hindrance on a molecular level. Under the conditions of this study, complement was found not to play a role in the inhibition of urease by specific antiserum.

Comparison of precipitin curves of antisera from x-radiated and nonradiated rabbits. Paul H. Maurer, David W. Talmage and Frank J. Dixon. *Dept. of Pathology, Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.*

As part of a study of the inhibition of immune response by radiation we have quantitatively analyzed the antibody formed by x-radiated and non-radiated rabbits immunized with bovine gamma globulin Fraction II (Armour). A study of the precipitin curves of both types of sera in the region of moderate antigen excess (inhibition zone) afforded a basis for comparison. Not ony were the sera obtained from some animals receiving 400 r 2 days to 5 hours before immunization low in antibody concentration but there was little inhibition of precipitation even at antigen concentrations of 50–90 times that of the equivalance zone. A few very low titer control sera behaved similarly. The sera will be compared mainly as to their immunochemical behavior from the equivalance zone through the region of maximum precipitation of nitrogen to one-third inhibition of precipitation. Analyses of the bovine gamma globulin by electrophoresis, supernatant tests, Oudin technique and immunologic tests for albumin demonstrated it to be homogenous. However, because of the possibility that gamma globulins might be immunologically heterogenous and thus account for the above observations, these results are being checked in experiments using crystalline bovine serum albumin as antigen. (This study was supported by Atomic Energy Commission contract No. AT(30-1)-1205.)

Further kinetic studies on immune hemolysis with special regard to the quantitative determination of hemolytic antibody. M. M. Mayer, C. C. Croft,* L. Levine* and A. A. Marucci.* *Dept. of Bacteriology, Johns Hopkins School of Hygiene and Public Health, Baltimore, Md.*

As indicated in previous studies, the kinetics of immune hemolysis in the presence of excess complement and limited amounts of antibody resemble those of enzymatic processes. Therefore, assays of hemolytic antibody should be based on velocity measurements rather than end point titrations. An assay method of this type has been developed and its validity has been studied extensively. Analyses have been found to be reproducible within ±5%.

Chemical studies on human erythrocyte receptor of influenza virus. J. F. McCrea (introduced by B. A. Briody). *Lister Inst. of Preventive Medicine, London, England.*

Methods of isolating influenza virus 'receptor-substance' from human erythrocytes were investigated. Method I: stroma were shaken with pentane until a gel formed; virus-inhibitory mucoid was isolated in good yield by extracting the gel with dilute ethanol. A stable readily soluble mucoprotein was finally obtained which inhibited hemagglutination by active and heated influenza A and B virus, but which showed negligible blood-group activity. The sedimentation diagram

revealed one slow-moving sharply defined component; three components appeared on electrophoresis. Two hexosamines, galactose, fucose, amino acids, and 4% of lipid were released on acid hydrolysis. In method II, modified from DeBurgh *et al.* (*J. Exper. Med.* 87: 1, 1948) stroma were heated with ether and the extracted lipomucoid purified by ethanol fractionation. The most active fraction showed two slow-moving components on electrophoresis and was markedly polydisperse in the ultracentrifuge. Active blood-group substance was present in all fractions. Method III was adapted from Hallauer's technique (*Zeits. f. Immunol.* 83: 114, 1934) of isolating blood-group substance by extracting dried erythrocytes in successive dilutions of alcohol. A small yield of ether- and water-soluble lipomucoid was obtained which actively inhibited virus hemagglutination. One sharply-defined component appeared in the ultracentrifuge. The same hexosamines and sugars were present as in method I, but the lipid content in methods II and III was approximately 5 times higher. Virus antihemagglutinin activity of all preparations was progressively destroyed on incubation with influenza virus or *V. cholerae* receptor-enzyme.

Behavior of viruses of Japanese and Russian encephalitis in Rhesus monkeys. J. ANTHONY MORRIS AND JOHN R. O'CONNOR.* *Dept. of Virus and Rickettsial Diseases, Army Med. Service Grad. School, Washington, D. C., and U. S. Fish and Wildlife Service, Laurel, Md.*

Twelve monkeys were inoculated intramuscularly with the Taira strain of Japanese encephalitis. None of the monkeys developed overt disease but 6 developed viremia. When challenged intracerebrally 61 days later, 6 of the monkeys survived, 4 of whom had shown viremia. No positive relation was demonstrated between the occurrence of viremia and the development of antibodies. Monkeys which developed significant amounts of neutralizing or complement-fixing antibody appeared to be more resistant to the intracerebral challenge. Nineteen monkeys were inoculated intramuscularly with varying amounts of the Moscow strain of Russian encephalitis. Viremia developed in 12 monkeys 2 of which had fever. Those given less than $10^{5.5}$ mouse intracerebral LD_{50} failed to develop viremia. Sixty-nine days later they were challenged by the intracerebral route. Only 2 of those who had shown viremia died while 6 of the 7 without viremia succumbed. There was a positive relation between the occurrence of viremia following peripheral inoculation and the development of complement-fixing and neutralizing antibodies. Moreover, those monkeys which had a neutralizing index greater than 100 at the time of challenge were resistant and those with complement-fixing antibodies at levels of $\frac{1}{16}$ or greater were solidly immune. Thirteen monkeys were vaccinated with formalinized Russian vaccine. Twenty-one days later when challenged by intramuscular injection of the Russian virus, 7 developed viremia indicating that the vaccination did not evoke sufficient antibody production to protect against viremia.

Serum bactericidal activity by turbidimetric growth assay method. LOUIS H. MUSCHEL* AND HENRY P. TREFFERS. *Dept. of Microbiology, Yale Univ., New Haven, Conn.*

This method involves the addition of broth after a suitable reaction period to tubes containing organisms, serum and complement. The tubes are then incubated until a readable density of growth is obtained. The optical density of each tube is directly proportional to the relative number of organisms surviving the bactericidal action of serum and complement. This method has been applied to the quantitative estimation of bactericidal antibody in immune and normal sera. Comparison of the growth densities with those of control tubes of complement alone permits calculation of the percentage kill of the organisms. In tests with *S. typhosa*, a linear relationship has been obtained between the probit kill and the logarithm of the serum, yielding by inspection the amount of serum required for 50% kill. Serum titers were found to be inversely proportional to the number of organisms employed. Using optically standardized suspensions of organisms, great reproducibility has been attained with different lots of complement, although, because of the many variables involved, titers obtained relative to a standard serum are preferable. Complement may be titered in similar manner using constant amounts of immune serum and organisms and varying amounts of complement. The activating effect of magnesium ion on this bactericidal reaction has been demonstrated. A linear relationship between immune serum and the ratio, immune serum/complement required for 50% killing of organisms, has been established which parallels that reported by Kent for hemolysin and complement.

Purification of serologically active principles, particularly protein and polysaccharides, from *Trypanosoma cruzi*. ARDZROONY PACKCHANIAN. *Dept. of Bacteriology and Lab. of Microbiology, School of Medicine Univ. of Texas, Galveston.*

Both the protein and the polysaccharide complexes were isolated from 3 grams of powder of *Tr. cruzi* according to Sevag's method with the following results: *Fraction A* (protein complex)—on the basis of Kjeldahl nitrogen, this sample contained 0.255% protein. Spectrophotometric analysis showed the presence of non-dialyzable

purine derivatives, such as d-ribose and desoxyribonucleic acid. In the Molisch test, this fraction showed a trace of carbohydrate detectable within a 30-minute period. *Fraction B* (protein)—on the basis of Kjeldahl nitrogen, this sample contained 0.044% protein. Spectrophotometrically, the fraction was free from purines, thus indicating the absence of nucleic acids. The Molisch test was negative for carbohydrates even after an overnight reaction period. *Fraction C* (polysaccharide complex)—on the basis of Kjeldahl nitrogen, this sample showed the presence of 0.033% protein. Spectrophotometric analysis showed the presence of non-dialyzable purine derivatives. The Molisch test was positive for carbohydrates. Since the primary aim was to characterize the nature of the components, no effort was made to determine the absolute amounts of each component in dry trypanosomes. The experimental data show that all 3 fractions are serologically active. The fact that Fraction B appears to be free from detectable carbohydrate and also the fact that Fraction C is presumably free from proteins indicate that both the protein and the polysaccharide components of trypanosomes are serologically active.

Antigenic properties associated with Arlacel-A (mannide oleate). PHILIP Y. PATERSON,* BETTY SMITHWICK* AND LEWIS THOMAS. *Dept. of Medicine, Tulane Univ. School of Medicine, New Orleans, La., and the Dept. of Virus and Rickettsial Diseases, Army Med. Service Grad. School, Washington, D. C.*

Dogs and rabbits, following vaccination with homologous brain suspended in the adjuvant mixture of Freund, developed a complement-fixing antibody which reacted in a specific manner with brain extracts. In addition the sera of these animals were found to react in the complement-fixation test with one of the adjuvant components, namely Arlacel-A. Dogs and rabbits were inoculated with a mixture of Bayol-F containing heat killed Mycobacterium tuberculosis and Lot #3375 of Arlacel-A. The post-vaccination sera of these animals reacted in the complement-fixation test at high dilutions with alcoholic solutions of the same lot of Arlacel-A. Dog and rabbit sera which reacted with Arlacel-A from Lot #3375 did not cross react with 5 different lots of Arlacel-A (Lots #3589, 3602, 3611, 3618 and 3534). The sera of rabbits vaccinated with Arlacel-A alone (Lot #3375) had no such serological activity. Two lots of Arlacel-A (Lots #3375 and 3618) without added Bayol-F and M. tuberculosis were found capable of sensitizing guinea pigs and eliciting severe anaphylactic reactions following challenge with either of the lots. These studies would indicate that the antigenicity of Arlacel-A preparations is probably due to some contaminant, rather than mannide oleate possessing antigenic properties itself.

Inhibition of mouse encephalomyelitis virus, in vitro, by certain nucleoprotein derivatives. HAROLD E. PEARSON, DONALD W. VISSER* AND DOROTHY L. LAGERBORG.* *Depts. of Med. Microbiology and Biochemistry, Univ. of Southern California School of Medicine, and Lab. Div., Los Angeles County Hosp., Los Angeles, Calif.*

Previous work showed that various substances including heavy metals, enzyme inhibitors such as dinitrophenol, cyanide, azide and iodoacetate, as well as certain amino acids inhibit the propagation of Theiler's GDVII strain of murine encephalomyelitis virus in tissue cultures of 1-day-old mouse brain contained in flasks of Simms' solution and incubated at 35° C. for 2 days. The present study revealed that adenine, thymine, adenosine, guanosine and cytidine inhibit virus propagation. Guanine, uridine, uracil, guanylic or cytidylic acids did not inhibit. Various substituted nucleosides including amino-, chloro-, diazo-, formamido-, hydroxy and methyluridine were inhibitory. Uridine partially reversed this inhibition. The 5-chlorouracil, ribosylthymine and glucosylthymine did not inhibit; 2,6 diaminopurine did inhibit; 5-chlorouridine was not effective against this viral infection of mice *in vivo*.

In vitro purification of anti-tissue antibodies. DAVID PRESSMAN AND LEONHARD KORNGOLD.* *Sloan-Kettering Inst., New York City.*

Anti-rat-liver, -kidney and -lung antibodies were purified *in vitro* by absorbing the radioiodinated globulin fraction of the antiserum with the saline insoluble portions of a tissue homogenate and then eluting the adsorbed radioantibodies with alkali at pH 11 or by heating to 65°. The fraction of the activity which localized was increased 5- to 10-fold. The localization properties of the eluates depended on both the original serum and the tissue used for purifications. There are cross reacting substances in the 3 tissues, but lung seems to contain materials which are unique to lung. By separating liver and lung tissues into two fractions (i.e. one rich in large blood vessels and the other in cells free of connective tissue), we found that the cross reacting components responsible for localization in the kidney and liver are in the blood vessels of the organs. With anti-lung serum, the lung localizing activity was found in greater concentration in the eluate from the loose cell fractions. The experiments indicate that the localization in lung may proceed by a different mechanism from the localization observed in liver and kidney.

Purification of lethal toxin of *Clostridium novyi*. JACOB J. PRUZANSKY (introduced by LOUIS PILLEMER). *Inst. of Pathology, Western Reserve Univ., Cleveland, Ohio.*

The solubility of the lethal toxin of *Cl. novyi* under varying conditions of pH, ionic strength, alcohol concentration, protein concentration and temperature has been determined. On the basis of these studies the lethal toxin in culture filtrates of *Cl. novyi* was carried through several stages of purification to produce in good yield a relatively stable preparation of high potency. The details of the purification of the lethal toxin and other pertinent data will be presented.

Purification and immunochemical characterization of alcohol-soluble, heat-stable hemolytic antibody-inhibiting hapten from sheep erythrocytes. H. J. RAPP,* F. A. H. RICE* AND M. M. MAYER. *Dept. of Bacteriology, Johns Hopkins School of Hygiene and Public Health, Baltimore, Md.*

An alcohol-soluble, heat-stable hapten from sheep erythrocytes has been extracted and purified. This hapten combines specifically with hemolytic antibody to sheep red cells prepared by immunization of rabbits with boiled sheep red blood cell stromata. Following extraction of the hapten from sheep red blood cell stromata with methanol, considerable purification has been achieved by fractionation with various organic solvents; the specific activity of the purified hapten is approximately 10–20 times as great as that of the crude initial alcohol extract, as measured by a precise, spectrophotometric hemolysis-inhibition assay. The hapten appears to be a lipid containing approximately 1% phosphorus and 2.5% nitrogen. It gives a doubtful or negative Molisch reaction, as well as negative biuret and Liebermann-Burchard tests. The substance is soluble in methanol, ethanol, iso-propanol, *n*-butanol, chloroform and hot ethylacetate. It forms stable emulsions in aqueous media. Emulsions prepared in saline flocculate on admixture with hemolytic antiserum, and this immune aggregation reaction has been studied quantitatively by analyses for uptake of antibody nitrogen. Hemolytic antibody as well as the hapten can be dissociated from washed aggregates.

Change in allergenicity of modified footstuffs, determined in man. BRET RATNER, SAMUEL UNTRACHT* AND CECIL COLLINS-WILLIAMS.* *Depts. of Pediatrics and Immunology, New York Med. College, New York City.*

We demonstrated in animal experiments that certain heat-treated and processed foodstuffs were reduced in anaphylactogenicity. We believe the following method offers an objective approach to the problem in the human subject. Skin sites in normal recipients are passively sensitized with serum from allergic donors with known food sensitivities to demonstrate the presence of allergic antibodies. Twenty-four to 48 hours later the recipient ingests the food under investigation in heat-denatured or processed forms. If no reaction ensues in the passively sensitized areas, the recipient then ingests the food in its native form after varying intervals. The absence of a reaction from the ingestion of food in modified form and the subsequent presence of a reaction following the ingestion of the same food in native form indicates that some change has occurred in the allergenicity of the foodstuff resulting from heat treatment or other processing. Preliminary experiments show that the albumin and globulin fractions of hard-boiled egg white appear to lose their allergenicity, but this does not hold entirely for the ovomucoid fraction. Cod liver oil was shown to have no allergenicity, whereas boiled cod fish still retains this quality. Peanut butter was found to be highly allergenic, whereas penaut oil is devoid of this property. We believe the dual ingestion procedure coupled with the Prausnitz-Kustner method offers a new approach to the study in man of the influence that various modifications of food proteins have on their allergenicity and passage through the intestinal wall.

Inhibitory effects of a fraction from human sputum on influenza virus. HARRY M. ROSE. *Dept. of Bacteriology, College of Physicians and Surgeons, Columbia Univ., New York City.*

It has been shown previously that human sputum contains substances other than specific antibodies which are capable of inhibiting hemagglutination by influenza virus (PR8 and Lee strains) and also of reducing the infectivity of these viruses for mice and chick embryos. Recent studies indicate that a potent antiviral fraction may be isolated from sputum by procedures which include separation in the electrophoresis-convection apparatus of Kirkwood *et al.* Chick embryos treated with doses of this fraction as late as 3–6 hours after infection with influenza virus show a marked decrease in the amount of virus present in their chorioallantoic membranes and fluids, as compared with controls. Moreover, mice survive and fail to develop obvious pulmonary lesions following the intranasal inoculation of mixtures containing equal amounts of inhibitor fraction and virus (up to 10,000 LD_{50}). The virus is capable of multiplying in the lungs of the animals but its behavior under the influence of the inhibitor bears a resemblance to that of unadapted virus.

Purification of lecithinase (alpha toxin) of *Clostridium welchii*. FLORENCE ROTH,* K. C. ROB-

BINS* AND LOUIS PILLEMER. *Inst. of Pathology, Western Reserve Univ., Cleveland, Ohio.*

The solubility of lecithinase (alpha toxin) in the filtrates of *Cl. welchii* have been determined in methanol-water and water mixtures of controlled pH, protein concentration, ionic strength and temperature. On the basis of these determinations, 4 steps have been developed for the separation of the lecithinase from other protein components of the filtrates. Purified lecithinase has been prepared containing over 2500 Lb/mg. of protein nitrogen. Details of fractionation procedures will be presented.

Possible relationships of epidemic keratoconjunctivitis virus to other viruses. ISAAC RUCHMAN AND WILLIAM F. DANIELS.* *Dept. of Bacteriology, Univ. of Cincinnati College of Med., Cincinnati, Ohio.*

That available strains of epidemic keratoconjunctivitis (EK) virus were indistinguishable from St. Louis encephalitis (SLE) virus had been established by cross-neutralization and cross-immunity tests. Infectivity tests in laboratory animals, however, indicated a difference. Since known immune sera to identify the EK virus do not exist, the sera from 14 patients who had recovered from EK infection were examined for the presence of neutralizing antibodies. Nine of the sera were negative for antibodies against both the EK and SLE viruses. Four sera had antibodies against both viruses and in all of them the neutralization index was higher against the EK virus. One serum had antibodies against the EK virus only. Further neutralization tests with sera from convalescent animals confirmed the serological identity of the two viruses. In addition 2 EK sera which neutralized the homologous virus either failed to neutralize the SLE virus or did so to a considerably lesser degree. Two other lines of the EK virus from the same source did not yield any additional information. However, one line of the same origin but received from a different source yielded a low titer which was consistent with that described for the classical EK strain. Nevertheless cross-neutralization and cross-immunity tests in animals established this virus as a strain of herpes simplex. This was confirmed histologically. Since a relationship was reported to exist between the EK and herpes viruses, cross-neutralization and cross-immunity tests between these two viruses were performed but no such relationship was found.

Immunization of monkeys with poliomyelitis viruses grown in cultures of monkey testicular tissue. JONAS E. SALK, L. JAMES LEWIS,* BRYON L. BENNETT* AND J. S. YOUNGNER. *Virus Research Lab. Univ. of Pittsburgh School of Medicine, Pittsburgh, Pa.*

Suspensions of tissue derived from mice or monkeys paralyzed by poliomyelitis viruses are considered undesirable as a source of virus for vaccines that might be tested in man because of the presence of CNS substance. The demonstration by Enders, Weller and Robbins that these viruses can be propagated in cultures of non-neural tissue has eliminated this objectionable characteristic of poliomyelitis virus suspensions. Using virus derived from roller tube cultures of human embryonic tissue or monkey testicular tissue, studies were undertaken to determine the relative antigenic activity of culture virus as compared with virus contained in animal CNS tissue. This was done by inoculation of monkeys with graded dilutions of virus suspension mixed with mineral-oil adjuvant to determine the antigenic extinction dilution as well as the level of antibody induced and its persistence. Comparison of virus with and without emulsification with mineral oil confirmed further the value of the adjuvant for enhancing antibody response. The comparability in antigenic activity of CNS virus and tissue culture virus of each of the three immunologic types has been established in a series of quantitative experiments. It has been observed also that differences in antigenic capacity exist among different strains cultivated in tissue culture; some appear to be better antigens than others. Culture virus treated with formalin in minimal concentrations sufficient to destroy infectious activity was found to be antigenic. The quantitative aspects of the destruction of infectivity and the retention of antigenicity are under investigation.

Quantitative and morphological aspects of 'incomplete' growth cycle of influenza virus in mouse brain. R. WALTER SCHLESINGER AND GEORGES H. WERNER (introduced by GEORGE K. HIRST). *Divs. of Infectious Diseases and Applied Immunology, Public Health Research Inst. of the City of New York, New York City.*

The conditions under which intracerebral inoculation of non-neurotropic influenza virus is followed by a single-step increase of viral hemagglutinin and complement-fixing antigen without commensurate rise in infectious titer have been studied further. The biological and morphological characteristics of 'incomplete' virus from mouse brain have been compared with those of mature virus derived from standard passages in eggs and of predominantly 'incomplete' virus prepared in eggs according to von Magnus. The two forms of non-infectious hemagglutinin appear to be similar in nature. Electron microscopy has revealed that the numbers of particles adsorbed on laked fowl erythrocytes are closely correlated with the hemagglutinin titers but bear no relationship to widely divergent infectious titers. Although the average size of particles from mouse brains har-

vested after a complete growth cycle is similar to that of mature virus from allantoic fluid, sizes of the former cover a wider range in both directions. Many of the adsorbed particles are large (about 250 mμ), flat, doughnut-shaped, suggesting the appearance of 'ghosts.' Comparable particles are absent from mouse brains harvested 1 to 3 hours after inoculation, i.e. before rise in hemagglutinin, from normal brains, or from brains infected with Western equine encephalomyelitis virus.

Enzymatic aspect of catalase-anticatalse reaction. M. G. Sevag and M. Zacco.* *Dept. of Microbiology, Univ. of Pennsylvania Med. School, Philadelphia.*

It has been shown that homologous antibody inhibits the activity of an enzyme containing a reversibly dissociating coenzyme group: hexokinase, d-glyceraldehyde dehydrogenase, pyruvic reductase, carboxylase, etc. It has also been known that heme-reacting anti-haptenic antibody is absent in immune sera against hemoglobin and other heme-containing antigens. The question, however, of whether or not the homologous antibody combining with the protein moiety of catalase can inactivate the non-dissociating heme has not been adequately investigated. Rabbits were immunized against crystalline beef liver catalase (generous gift of Worthington Biochemical Laboratory, Freehold, N. J.) by alternating subcutaneous and intravenous injections every 3rd day. Using 5 mg. catalase per injection, a total of 20 injections was made. Catalase in dilutions up to 1:400,000 reacted with anticatalase sera. One ml. of serum completely precipitated an amount of catalase which decomposed 14.5 ml. of 0.5 M hydrogen peroxide. Centrifuged supernatant was inactive. The precipitate was washed twice in the cold with 1 ml. of immune serum each time; the washings were practically inactive. Washed catalase-anticatalase precipitate exercised the original activity. The activity was determined *a*) manometrically at 37° C. using 1 ml. of 1:10,000 suspension of the precipitate in immune serum, and *b*) at 5° C. (to prevent dissociation) using the bulk of the washed precipitate and titrating the excess hydrogen peroxide iodometrically. These findings show that catalase-anticatalase combination fails to inactivate the catalysis by the heme group, and in this combination antibody fails to form a mechanical barrier against the mutual affinities of the active group and hydrogen peroxide.

Hemolysis with complement of intact azo-erythrocytes sensitized with antisera homologous to attached azo-grouping. Arthur M. Silverstein* and Frank Maltaner. *Div. Labs. and Research, N. Y. State Dept. Health, Sloan-Kettering Inst. for Cancer Research, New York City.*

Sheep erythrocytes which had been coupled with a diazotized solution of *p*-arsanilic acid were hemolyzed with complement after sensitization with anti-beef-protein-*p*-azo-phenyl-arsonic acid rabbit serum. No hemolysis was observed upon sensitization with a heterologous control serum specific for the *p*-azo-benzoic acid grouping. The number of sites on the erythrocyte specific for sensitization could be changed by varying the intensity of coupling of the diazonium salt solution to the cells. The azo-erythrocyte preparation reacted with the antiserum specific for the azo-grouping in the same manner as normal erythrocytes react with the specific anti-erythrocyte antibody, or hemolysin. Thus the degree of hemolysis of a constant amount of azo-erythrocytes varied with changes in the concentration of either antiserum or complement. (Supported in part by a cancer control grant from the National Cancer Institute of the National Institutes of Health, U. S. Public Health Service.)

Effect of ACTH, streptomycin, ACTH and streptomycin on experimental bovine tuberculosis in the rabbit. David T. Smith and James M. Bacos.* *Dept. of Bacteriology Duke Univ. School of Med., Durham, N. C.*

Rabbits were sensitized to tuberculin by a subcutaneous injection of R_1 tubercle bacilli. Two months after the sensatizing injection 4 mg. of a virulent strain of bovine tubercle bacilli, in 0.25 mm. of physiological saline, was introduced into the trachea with a needle and allowed to flow into the right upper lobe. Each of 10 rabbits was given 2.5 units of ACTH, q 6h, or a total of 10 units daily for 10 days; 2.5 units, q 12h, or 5 units daily for 10 days; 2.5 units q 24h for 10 days; and 1.25 units daily for 94 days. Each of 10 rabbits received 15 mg. of Dihydrostreptomycin, q 6h, or a total of 60 mg. daily for 10 days; and 30 mg. q 12h or a total of 60 mg. for 10 days; and 30 mg. daily for 104 days. Each of 10 rabbits were given simultaneously both ACTH and Dihydrostreptomycin therapy. The 4th group of rabbits were not treated. Three rabbits from the control group died of tuberculosis between the 76th and 91st day and the remaining 7 had extensive disease when sacrificed on the 124th day. One rabbit from the ACTH group died on the 50th day of unknown cause. In the remaining 9 the disease was comparable to that in the controls. Six of the 10 rabbits receiving Dihydrostreptomycin showed no gross evidence of infection but 4 had about 50% as much disease as the controls. Eight of the 10 rabbits receiving the combined ACTH and Dihydrostreptomycin treatment showed no gross evidence of disease. Each of the remaining 2 animals had one small area of infection. In another group of 24 rabbits, killed on the 4th day after infection, a Herxheimer-like reaction was found in the rabbits, being treated by

Dihydrostreptomycin but not in the controls, ACTH or combined ACTH and Dihydrostreptomycin groups.

Effect of cortisone on infectious fibroma and infectious myxoma of rabbits. MARGARET H. D. SMITH. *Dept. of Pediatrics, Tulane Univ. School of Medicine, New Orleans, La.*

Rabbits injected with cortisone in doses of 2.5–60 mg. daily over a 2- to 3-week period developed much larger tumors upon inoculation with *fibroma* virus than the control animals receiving virus but no cortisone. Within this range, the larger the dose of cortisone, the larger the tumor. This effect upon the tumor was present only if the daily cortisone injections were begun before appearance of the tumor. If cortisone was delayed until appearance of the tumor, tumor size was affected little or not at all. Metastases were not observed. There was no striking difference in the amount of virus demonstrable per gram of tumor weight. The histological changes will be discussed; also the character of the cortisone-fibroma tumors when subjected to further rabbit passage. In the case of *myxoma*, rabbits receiving cortisone prior to virus inoculation displayed a striking absence of the malaise and anorexia characteristic of the disease, also much less mucopurulent discharge from the mucous membranes than usual. Death occurred after approximately the same interval in both cortisone-treated and control rabbits.

Neutralizing antibodies against recently isolated viruses in sera of human beings. K. C. SMITHBURN. *Labs. of Div. of Med. and Public Health, Rockefeller Fndn., New York City.*

Sera from 297 indigenous residents of British East Africa, each previously tested against yellow fever virus, were tested for their capacity to neutralize 8 different neurotropic viruses, namely: Bwamba fever, West Nile, Semliki Forest, Bunyamwera, Ntaya, Mengo (= E-M-C group), Zika, and Uganda S, all isolated in Uganda. Only 3 of these viruses have ever been isolated from human beings. The others were recovered from naturally infected mosquitoes and wild animals. The purpose of the study was to determine the frequency with which these agents attack man. The results indicate that infection in man with Mengo virus is probably uncommon in the localities sampled. Neutralizing antibody against all the others occurred with sufficient frequency to indicate that infection with them in human beings is not uncommon. More than 40% of the sera neutralized Bwamba fever virus. The specificity of the results is established by the fact that many sera showed no neutralizing antibody for any of the viruses and by the fact that every serum was negative for at least 3 viruses. Results with each agent served as controls for the tests with the others. A number of the sera neutralized more than 1 virus. Analysis of the data pertaining to these sera suggests differences in the modes of transmission of the viruses concerned.

Action of methionine in viral infections. DOUGLAS H. SPRUNT, JOHN L. WOOD* AND H. L. SCHUMAN.* *Inst. of Pathology and Div. of Chemsitry, Univ. of Tennessee, Memphis.*

In earlier experiments we have shown that a low protein diet for a time increases the resistance of mice to swine influenza. Methionine was found to alter this so that the mice became more susceptible to swine influenza. The possibility that this change was due to an increased metabolism of the proteins in the liver was suggested. In order to study the effect of methionine in viral infections, methionine, in which the sulphur was radioactive, was given to mice on a low protein and a control diet. The animals on a low protein diet for 2 weeks which were inoculated with swine influenza were found to have more radioactive sulphur in the lungs and liver than did the control animals. These results suggest the two possibilities; *1)* that methionine is concerned with viral multiplication as is indicated by the increase of methionine in the lung; *2)* the metabolism of protein in the liver may be altered as is indicated by the increase of methionine in the liver of mice on a low protein diet with viral infections. This change in protein metabolism may have decreased antibody production and thus lowered the resistance of the mouse to infection.

Role of popliteal lymph node cells and splenic cells in synthesis of diphtheria antitoxin. ABRAM B. STAVITSKY. *Dept. of Microbiology, Western Reserve Univ. Med. School, Cleveland, Ohio.*

The data presented implicate the lymph node cells in antitoxin production following local injections and the splenic cells after intravenous injections of toxoid. Removal of the node 7 days before the second local injection of toxoid results in a greatly reduced secondary antitoxic response compared to that of the unoperated controls. Likewise splenectomy 1 week before the second intravenous injection led to a considerably reduced antibody response. Intravenous injection into normal rabbits of lymph node cells removed about 6 days after the second local toxoid injection resulted in the appearance of up to 1 unit of antitoxin per ml. of blood in the recipient 5 days later. Similar results followed the transfer of splenic cells from rabbits which were intravenously inoculated with toxoid. At any one time following the appearance of antitoxin there was much more total and antibody protein in the blood of the recipients than in the transferred cells. Before transfer the cells contained enough antigen to produce several units of antitoxin upon injection into primarily immunized rabbits, but not enough

to immunize normal rabbits primarily. Thus normal rabbits which received lymph node or splenic cells from immunized animals developed antitoxin 5 days later but did not display a secondary antitoxic reaction to an injection of toxoid 2 or 3 weeks later. The available evidence suggests that the transferred cells either synthesize or cause the synthesis of antitoxin *de novo* in their new host.

Immunologic activity of acid-precipitate in hemolytic streptococcus filtrates (NY5 strain). AARON H. STOCK AND ETHEL VERNEY.* *Dept. of Bacteriology and Immunology, Univ. of Pittsburgh School of Med., and Children's Hospital, Pittsburgh, Pa.*

In 1930 Ando and associates reported on an acid-precipitable fraction in filtrates of cultures of hemolytic streptococci. They believed the fraction was a nucleoprotein, bacterial in origin, and a cause of false-positive Dick tests. Ando *et al.* used 'ordinary' broth in their work. Since either of the usual ingredients of broth may contain acid-precipitable substances, the existence of the bacterial entity was questionable By utilizing filtrates from cultures grown in dialyzate broths, we have demonstrated an acid-precipitable fraction that unquestionably developed after bacterial growth. Immunologic studies were made with several preparations of acid-precipitate. It was found that scarlet fever toxin was adsorbed on some of the acid-precipitates. None of the materials tested appeared to give false-positive Dick tests. The results of serologic tests against normal serums, and antibacterial and antitoxic serums of streptococcal origin will be presen ted.

Poliomyelitis viruses propagated in tissue cultures as antigens in the complement fixation test. ARNE SVEDMYR,* JOHN F. ENDERS AND ANN HOLLOWAY.* *Research Div. of Infectious Diseases, Children's Med. Center, Boston, Mass.*

Complement fixation, apparently specific, was obtained with antigens containing Lansing and Brunhilde viruses and their homologous antisera prepared by the hyperimmunization of monkeys with infected monkey cord. Moreover, a significant rise in antibody titer against Brunhilde antigen was demonstrated in the convalescent serum of several patients from whom Brunhilde type viruses had been isolated. The drop technique of Fulton and Dumbell (*J. Gen. Microbiol.* 3: 7, 1949) was used. The antigens were concentrated from supernatant fluids of human tissue cultures by means of a simple method of ultrafiltration (SEIBERT, *J. Biol. Chem.* 78: 345, 1928) and clarified by centrifugation at high speed. Anticomplementary activity was abolished by moderate heating. Materials concentrated as much as 600 times have been used as antigens. A Brunhilde antigen in supernatant fluids of suspended cell flask cultures, showed optimal activity when concentrated only 20 times. On the other hand certain fluids infected with the Lansing strain have required a greater degree of concentration. The best starting materials for the preparation of antigens have so far been obtained from roller cultures in which a simple dialyzable medium was substituted for a complete medium after cell growth had taken place and after inoculation of the cultures with the virus.

The cockroach as a natural extrahuman source of poliomyelitis virus. JEROME T. SYVERTON, ROBERT G. FISCHER,* SIDNEY A. SMITH,* RICHARD P. DOW* AND HERBERT F. SCHOOF.* *Dept. of Bacteriology and Immunology, Univ. of Minnesota and the Communicable Disease Center, U. S. Public Health Service, Atlanta, Ga.*

Studies in this laboratory have made known the ability of the cockroach *Periplaneta americana*, under laboratory conditions to acquire, maintain and excrete *1*) mouse encephalomyelitic virus, GDVII strain; *2*) Coxsackie virus, group A, type 4; *3*) human poliomyelitis virus, Brunhilde type, Minnesota and Mahoney strains. Sufficient virus was excreted daily over a period of from 7–15 days to paralyze and more commonly to kill the recipient animals. The identity of each virus after recovery from recipient animals was established. These laboratory findings were extended by field studies to learn whether cockroaches captured under natural conditions will yield on test in susceptible hosts human poliomyelitis virus and/or Coxsackie virus. It is timely to report the isolation of 4 strains of poliomyelitis virus from 4 lots of cockroaches captured on the premises of paralytic poliomyelitis patients from 2 states. Cockroaches from a 3rd state are under study. These lots of cockroaches were made up of 3 species commonly associated with man: *Periplaneta americana*, the large winged American cockroach; *Supella supellectilium*, the tropical roach; *Blattella germanica*, the common small German Roach or 'croton bug.' The premises from which the roaches were obtained are representative of good and poor sanitary environments. It should be pointed out that these results are preliminary to studies under way and that the relation of the cockroach to the natural transmission and dissemination of poliomyelitis remains to be determined. As far as we are aware this is the first time that a cockroach has been implicated as a natural vector of a virus.

Experimental disseminated encephalomyelitis produced in mice by proteolipide A and B (Folch and Lees). CHLOE TAL* AND PETER K. OLITSKY. *Rockefeller Inst. for Med. Research, New York City.*

Mice, especially the H-line, Swiss-W strain, useful for experimental production of disseminated encephalomyelitis (allergic or isoallergic enceph-

alomyelitis) (Olitsky and Yager, *J. Exper. Med.* 90: 213, 1949), have been injected with several chemical substances and with different fractions of homologous and heterologous cerebral tissue, all admixed with the Freund type of adjuvant. The object of the tests was to identify or isolate the encephalitogenic agent; but they failed. A new group of lipoproteins, the proteolipides, discovered recently by Folch and Lees (*J. Biol. Chem.* 191: 807, 1951) were then used. Proteolipide A, prepared by the latter from cattle brain and made available by their generosity, when mixed with adjuvant was found to be characteristically but mildly encephalitogenic in 7 of 20 mice. Proteolipide A and B prepared in this laboratory from normal adult mouse brain (mice react more regularly to homologous brain tissue), added to adjuvant, induced disseminated encephalomyelitis in 27 of 28 mice receiving it. What is significant is that the residue of brain tissue was practically inactive. It is thus probable that the encephalitogenic agent is in the proteolipide fraction. Since this is not a pure substance, further study is being carried out to isolate, if possible, any active component.

Localization of x-ray injury to the initial phases of antibody response. W. H. Taliaferro, L. G. Taliaferro* and E. F. Janssen.* *Univ. of Chicago, Chicago, Ill.*

Hemolysin titers (50% units determined photometrically) were followed at frequent intervals in rabbits receiving total body x-irradiation with 600 or 700 r before or after a single injection of a standardized suspension of approximately 1.25 ml. of 1% sheep red cells/kg. rabbit. The administration of 700 r to rabbits during 1 week before the injection of red cells resulted in approximately doubling the induction period, decreasing the rate of the initial antibody rise, and reducing peak titer to about 1/7th that in nonirradiated controls. These effects disappeared after a month in that an essentially normal response was obtained in rabbits injected with red cells a month after irradiation. Similar irradiation of rabbits from 2 hours to 2 days after immunization also resulted in doubling the induction period but it did not decrease the peak titer as compared to controls. The lengthening of the induction period in both affected groups and the occurrence of normal amounts of antibody in animals irradiated when the immune process had been in progress only 2 hours indicate that the damage of x-irradiation to antibody formation is largely limited to the early phases of the immune process. (Work done under a contract between the U. S. Atomic Energy Commission and the University of Chicago.)

I^{131} labelled antigen precipitation as a measure of quantity and avidity of antibody. David W. Talmage* and Paul H. Maurer* (introduced by Frank J. Dixon). *Dept. of Pathology, Univ. of Pittsburgh School of Med., Pittsburgh, Pa.*

A modification of the labelled antigen precipitation method of Eisen and Keston has been used to survey over 300 antisera obtained in the study of x-radiation effect on antibody production (to bovine gamma globulin in rabbits). Where possible the results have been compared with those obtained by quantitative precipitin techniques. The amount of antibody was expressed in terms of micrograms of antigen nitrogen precipitated per ml. of serum at the point of 80% antigen precipitation; but these values, if desired, may be converted to antibody nitrogen using known antibody/antigen ratios. A plot of the percentage antigen precipitated versus micrograms antigen added per ml. serum gives a curve which reflects the ease with which precipitation is inhibited by excess antigen. The slope of this curve was found to vary markedly from serum to serum but remained relatively constant for any individual serum despite dilution in saline or normal serum. These differences in inhibition of precipitation reflect a basic quality of the antibody, most likely its avidity. The sensitivity of the method is limited only by the solubility of the specific precipitate at 0° C., which approximated one microgram antibody nitrogen per ml. The advantages of this method are: *1*) it consumes only 3–4 ml. of serum, *2*) it takes one-third to one-fifth of the time required of quantitative precipitin methods and *3*) it provides results which may be used either as a separate measure of antibody content or when desirable serve as a guide for quantitative precipitin determinations. (This study was supported by Atomic Energy Commission contract No. AT(30-1)-1205.)

Carditis in the generalized Shwartzman reaction produced with group A streptococci. Lewis Thomas, Floyd Denny and Joan Floyd. *Research Lab. of the Heart Hospital, Dept. of Pediatrics, Univ. of Minnesota Med. School, Minneapolis.*

Systemic infection of rabbits by Group A streptococci caused a marked alteration in the response to bacterial toxins capable of eliciting the Shwartzman reaction. Streptococcal infection was produced by an intravenous injection of broth suspensions of streptococci. Two or 3 days later the animals were given an intravenous injection of toxin derived from cultures of meningococci or *S. marcescens*. Death occurred within 5 days after the injection of toxin in approximately 50% of the animals, and lesions were demonstrable in the heart and kidneys in over 70%. The cardiac lesions varied depending on the duration of life after the injection of toxin. In animals dying within the first 24 hours, extensive hemorrhages into the

myocardium were seen. In animals dying or sacrificed between the 2nd and 8th day, the lesions consisted of *1*) fibrinoid deposition within the walls of blood vessels, with necrosis of the vessels, *2*) extensive necrosis of myofibers, *3*) pericarditis, *4*) mild valvulitis, and *5*) numerous collections of mononuclear inflammatory cells, 'owl-eyed' cells, and multinucleated giant cells. The kidney lesion was bilateral cortical necrosis. Control animals infected with streptococci without toxin, or given toxin without streptococcal infection, showed no cardiac or renal lesions. Penicillin treatment did not prevent the development of carditis unless instituted at the time of injection of streptococci. Studies on the capacity of heat-killed streptococci to produce similar cardiac and renal lesions are in progress.

Effect of heterocyclic and other thiosemicarbazones on vaccinia infection in the mouse. R. L. Thompson and S. A. Minton, Jr.* *Dept. of Microbiology, Indiana Univ. Med. Center, Indianapolis.*

Certain derivatives of thiosemicarbazone have been observed to protect mice against the vaccinia virus. Protection is obtained when the virus is inoculated intracerebrally and the compounds are administered in the diet. In experiments in which the I.H.D. strain of virus was used and benzaldehyde thiosemicarbazone was fed in a casein-sucrose diet in concentrations of from 0.02 to 0.08%, 144 of 244 treated mice (59%) survived in contrast to 40 of 243 untreated animals (16%). In similar experiments benzaldehyde semicarbazone (0.1%) failed to reduce the fatality rate. Substitutions in the benzene nucleus or in the 1- or 4-positions of the thiosemicarbazone portion of the molecule were found to reduce the protective capacity. The activity of cinchoninaldehyde, isatin and 2-thenaldehyde thiosemicarbazones was equal to or greater than that of benzaldehyde thiosemicarbazone; nicotinaldehyde and isonicotinaldehyde thiosemicarbazones were less active and 3-thenaldehyde thiosemicarbazone exhibited variable results.

Adaptive phenomena in a biosynthetic pathway. Henry J. Vogel* and Bernard D. Davis. *U. S. Public Health Service Tuberculosis Research Lab., Cornell Univ. Med. College, New York City.*

It has been suggested by Monod, Stanier and others that the formation of so-called constitutive enzymes, like that of adaptive enzymes, may involve participation of their substrates. Since the substrates of constitutive enzymes are normally present in the cell, this hypothesis seemed capable of being tested with bacterial mutants deficient in such a substrate. Accordingly, growth rate experiments were performed on the anabolic utilization of an ornithine precursor (I) (*Federation Proc.* 10: 423, 1951) by mutant 39A-23 of *Escherichia coli*. This strain is blocked before I, and hence will not grow on minimal medium, containing glucose and ammonium salts as carbon and nitrogen sources, unless supplemented with a member of the biosynthetic sequence: I, ornithine, citrulline, arginine. Cells grown on a limiting supplement of I, when subsequently inoculated into medium containing either 4 μg/ml. of L-arginine or an equimolar concentration of I, gave almost identical growth curves without appreciable lag. After preliminary growth on limiting arginine under otherwise identical conditions, approximately the same curve was obtained on arginine; in contrast, on I a pronounced lag was observed, although ultimately identical growth levels were attained. This effect suggests that one or more 'constitutive' enzymes on the anabolic pathway of I are formed adaptively in response to I. This interpretation has been confirmed by studies on the enzyme activity of non-growing cell suspensions. The same adaptive phenomena have not been demonstrable with certain other intermediates, including ornithine and citrulline.

Appearance of diphtheria antitoxin following transfer of cells taken from immunized rabbits. Odd A. Wager* and Merrill W. Chase. *Dept. of Microbiology, New York Univ. College of Medicine, and Rockefeller Inst. for Med. Research, New York City.*

Because diphtheria antitoxin can be determined accurately and natural diphtheria antitoxin is practically absent from rabbits, the experiments reported by Chase (*Federation Proc.* 10: 404, 1951) and by Harris and Harris (*Federation Proc.* 10: 409, 1951) have been repeated with cells taken from rabbits undergoing active immunization with diphtheria toxoid. The report of the appearance of circulating antibody within a few days after normal animals were injected with cells from lymph nodes and spleens of immunized animals was confirmed. Diphtheria antitoxin was found as early as 18 hours after the intravenous injection of washed cells (0.5–1.8 ml.), the concentration ascending until the 5th day (from $\frac{1}{50}$ to $\frac{1}{4}$ unit antitoxin/ml.) and falling during the following 5 to 9 days. In these experiments corresponding antibody was not found in extracts of cells subjected to grinding. Cells were secured from donors between 8 and 10 days following first stimulation with toxoid or following a secondary stimulation in animals given their primary stimulus long before. In addition to the early appearance of antibody in the recipient animals, there was a separate event commencing at about the 12th day, a marked production of antibody reaching its peak around the 4th week and subsequently decreasing. This

latter effect is interpreted as active immunization indicative of the presence of antigen associated with the well-washed cells, a conclusion further suggested by the secondary response of the cell recipients to a much later injection of toxoid.

Poliomyelitis and Coxsackie viruses from Egyptian flies. ROBERT WARD. *Dept. of Pediatrics, New York Univ. College of Med., New York City.*

The so-called 'sanitation theory' of poliomyelitis holds that the people of countries having primitive sanitation become immunized early as a result of repeated exposure to virus. Serological evidence to support this is derived from studies showing the development of neutralizing antibody to the Lansing strain at an earlier age in endemic countries than obtains in areas where the disease is epidemic. A comparable difference is seen in the age incidence of the paralytic disease. On the other hand, in countries where epidemics occur there is less opportunity for constant widespread fecal (virus) dissemination with resulting delay of the natural immunizing process. One possible factor of importance in explaining the inception of epidemics during the warm season is the repeated finding of virus in flies in epidemic areas and their proven ability to contaminate food. Therefore it seemed important to determine whether or not poliomyelitis virus could be detected easily year in year out in flies trapped in a country where the disease is endemic. A start was made by collecting flies during the summer of 1951 in and around Cairo, Egypt. Although only a few specimens have been tested to date in monkeys, at least one has yielded poliomyelitis virus. Nine of 13 specimens tested in newborn mice have yielded Coxsackie-like viruses. The results of further tests and the implications of the findings will be discussed.

Cultivation of Coxsackie viruses in human tissues. T. H. WELLER, F. C. ROBBINS AND M. B. STODDARD (introduced by JOHN F. ENDERS). *Research Div. of Infectious Diseases, Children's Med. Center, Boston, Mass.*

Cultures of various human tissues have recently been used in this laboratory for the isolation of poliomyelitis viruses. The demonstration of the occasional coexistence of Coxsackie and poliomyelitis viruses in human feces prompted an investigation of the behavior of the former agents in tissue cultures. Both suspended cell and roller tube cultures of several human tissues were employed. The High Point strain of C virus was selected as a representative of Dalldorf's Group A and the DeMole strain as a representative of Group B. Evidence has been obtained that the DeMole strain may be propagated serially in suspended cell cultures of human kidney, brain, or intestinal tissue, but unlike the poliomyelitis viruses does not multiply in cultures of human embryonic skin-muscle tissue. Quantitative data on the multiplication of DeMole virus will be presented. No cytopathogenic effect of the DeMole virus similar to that exhibited by the viruses of poliomyelitis has so far been noted in roller tube cultures of human embryonic skin-muscle or kidney tissue. Comparable studies are in progress with the High Point strain. An additional strain of Coxsackie virus having the pathogenic properties of Group A was recovered from tissue cultures of human kidney inoculated with fecal material from a case of non-paralytic poliomyelitis. This agent has been propagated in cultures of human tissues and has induced a cytopathogenic effect.

Preparation and use of stroma of normal and trypsinized human red blood cells for quantitative hemagglutination studies. HENRY J. WINN,* MATTHEW C. DODD AND CLAUDE-STARR WRIGHT.* *Depts. of Medicine and Bacteriology, Ohio State Univ., Columbus, Ohio.*

Data from previous studies carried out in this laboratory indicated antiagenic differences between normal human erythrocytes (O, RH+) and cells treated with .1% trypsin for 30 minutes at 37° C. In the course of investigating the nature of these differences quantitative hemagglutinin determinations were carried out according to the methods of Heidelberger and Kendall (1942). Human erythrocytic stroma were found to be markedly soluble in saline controls buffered at pH 7.4 and data obtained are, therefore, unreliable. Attempts to prepare stroma which would be more satisfactory for such purposes have been carried out. Stroma have been prepared from normal cells and from cells previously treated with trypsin. Stroma from trypsinized cells are more readily freed of hemoglobin and show greater tendency to flocculate than do stroma from normal cells. Liquid nitrogen distilled water and mechanical disintegration have been employed to effect separation of hemoglobin from intact stroma which were subsequently subjected to a variety of washing procedures in which temperature, pH and salt concentration were controlled. Lysis of red blood cells with distilled water at pH 4.5 to 5.5 and low ionic strength at ice box temperature is most satisfactory. Solubility of such preparations in saline and normal rabbit serum buffered at pH 5.0 to pH 7.4 is increased at higher pH. Results of quantitative hemagglutinin determinations carried out in the lower pH range have been obtained and these are compared to initial results at pH 7.4.

A study of factors important for the application of roller tube cultures of monkey testicular tissue to quantitative immunologic studies with poliomyelitis viruses. J. S. YOUNGNER, JONAS E. SALK AND ELSIE N. WARD.* *Virus Research Lab., Univ. of Pittsburgh School of Med., Pittsburgh, Pa.*

The cytopathogenic indicator of poliomyelitis virus multiplication in tissue culture and the inhibitory effect of antiserum upon virus growth has provided the means for development of a useful, quantitative, immunologic tool. For these investigations the techniques described by Robbins, Enders and Weller have been modified; principal differences are the use of monkey testicular tissue rather than tissue of human origin, and minor differences in the composition of the nutrient media employed. In the course of these studies, marked differences among strains with respect to the degree of their cytopathogenic effect were observed. For this reason prototype viruses were selected as antigens for the serologic test from among strains that produce severe changes evident most consistently and appearing in the shortest time. Employing as prototype viruses three strains of marked cytopathogenic activity, i.e. Mahoney (Type 1), Y-SK (Type 2), and Saukett (Type 3), an attempt has been made to standardize a neutralization test for the determination of titer of serum antibody. Among factors studied for their influence upon neutralizing antibody titer were *1*) number of tissue culture infectious doses of virus, *2*) inoculation of serum-virus mixtures with or without preincubation and *3*) the use of one or more treatments with antiserum dilutions at the time of successive fluid changes. With a procedure involving the inoculation of serum only once, i.e. when mixed with virus, it is possible to know the titer of the serum in 6 days using approximately 10–32 $TCID_{50}$. The applications of the method will be illustrated.

ABSTRACTS RECEIVED LATE

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Serum and aortal lipid changes in atherosclerotic hens treated with adenosine-5-monophosphate. LAWRENCE J. MILCH,* ROBERT F. REDMOND,* WILLIAM W. CALHOUN,* HERMAN I. CHINN AND CARDIOVASCULAR RESEARCH GROUP. *Dept. of Pharmacology and Biochemistry, USAF School of Aviation Medicine, Randolph AFB, Tex.*

Harvill (private communication) had observed that adenosine-5-monophosphate (AMP) (MY-B-DEN, Ernst Bischoff Co., Ivoryton, Conn.), markedly depressed the serum cholesterol levels in men. In view of the presumed importance of cholesterol to the general problem of atherosclerosis, AMP was administered to 2-year old hens in which aortic atherosclerosis had spontaneously developed. It was found that this treatment in chickens not only depressed cholesterol levels but simultaneously elevated serum phospholipid values so that the phospholipid/cholesterol ratio was increased by 30%. In addition, the serum concentration of the S_f 12-20 class of lipoproteins, as determined ultracentrifugally, was increased in the AMP-treated hens by 300%. These serum changes were associated with an average decline of 50% in aortal cholesterol concentrations and an equally significant fall in aortal fat content. Corresponding to the chemical changes observed in the aortas of AMP treated hens, gross grading of the extent of atherosclerosis at autopsy indicated that AMP markedly reduced the pathology.

Variations of oxygen consumption and rectal temperature of dogs during nembutal anesthesia. GABRIEL G. NAHAS* AND ALLAN HEMINGWAY. *Dept. of Physiology, Univ. of Minnesota, Minneapolis.*

Eight mongrel dogs, weighing from 16–31 kg., were anesthetized with a standard dose of nembutal amounting to 30 mg/kg. of body weight. Using the open circuit method, oxygen uptake was measured 4 times during a period of 2 hours following onset of anesthesia. Continuous rectal temperature was also recorded, using a thermistor. The procedure was carried out in a constant temperature room set at 25° C. After 30 minutes of anesthesia, the average oxygen uptake fell from a preanesthetic control value of 160 cc/m²/min. to 115 cc., a 28% drop, while the average minute volume fell from 6.65 to 2.47 l., a 63% drop. During the same period, the average rectal temperature fell from 38.84° C. to 37.76° C. After 60 minutes of anesthesia, the average oxygen uptake was 128 cc/m²/min., the minute volume 4.0 l., and after 120 minutes, 172 cc. and 4.4 l. respectively. The animals started shivering between 80 and 100 minutes after the onset of anesthesia. The average rectal temperature after 2 hours of anesthesia was still 37.19° C., a value significantly lower than the preanesthetic control one. Attempts were made to determine if the animals were equally sensitive to hypoxia throughout the foregoing period of anesthesia.

AMERICAN SOCIETY OF BIOLOGICAL CHEMISTS

Action of fluoroacetate on serine and glycine metabolism in vivo. JORGE AWAPARA. *Univ. of Texas, M. D. Anderson Hosp. for Cancer Research, Houston.*

As a result of blocking the oxidation of citric

acid *in vivo* by the administration of non-lethal doses of sodium fluoroacetate, a series of changes has been observed in the distribution of free amino acids in various organs of the rat. Serine increases significantly in the heart of fasted rats 3 hours after the administration of 1.6 mg/kg. of sodium fluoroacetate. No changes were observed in the serine content of the liver when fluoroacetate was given alone, but the serine concentration doubled in the liver if pyruvate and fluoroacetate were given simultaneously. The administration of pyruvate or α-ketoglutonate by themselves did not elicit any changes in the serine content of the organs investigated. A similar observation was made on the glycine content of the heart and liver. The administration of fluoroacetate and pyruvate resulted in a significant increase in the glucine content of the heart, but not in the liver. The mechanism responsible for these changes in the fluoroacetate treated animals has not been elucidated as yet. The formation of serine from lactate or pyruvate has been reported by Buchanan, Sonne and Delluva (*J. Biol. Chem.* 173: 81, 1948) and Anker (*J. Biol. Chem.* 176: 1337, 1948). They postulated the direct formation of serine from pyruvate or lactate, prior to conversion to glycine. In the present study, a defective pyruvate oxidation could conceivably lead to an alternate pathway, namely serine, and/or glycine formation.

Metabolism of 2-aminofluorine in rat. HELMUT R. GUTMANN, GLADYS E. KIELY AND MICHAEL KLEIN (introduced by JOHN L. WOOD). *Cancer Research Labs., Univ. of Florida, Gainesville.*

Using diazotization and coupling with sodium 2-naphthol-3,6-disulfonate (R-salt), the distribution of aromatic amino groups in the tissues of normal, riboflavin depleted and partially hepatectomized rats has been studied 4 and 12 hours following a single intraperitoneal injection of the carcinogen, 2-aminofluorene. In all instances the amounts of diazotizable compound which could be recovered decreased with time as described previously for 2-acetylaminofluorene. On the basis of the properties of diazotized and coupled 2-amino-7-hydroxyfluorene it is suggested that the apparent *in vivo* destruction of injected diazotizable material may be due to hydroxylation of 2-aminofluorene. No appreciable differences in the distribution of administered diazotizable compound were observed between normal and riboflavin deficient rats. On the other hand, the quantities of diazotizable material in the tissues of hepatectomized rats were 2–3 times greater than in intact animals. The data indicate that the liver is involved in the apparent decrease of the amino group of injected 2-aminofluorene.

Effect of stilbestrol on body weight and reproductive organs of male rat. D. W. SNAIR,* SYBIL E. JAFFRAY,* L. I. PUGSLEY AND L. A. SCHWINGHAMER.* *Food and Drug Lab., Dept. of Natl. Health and Welfare, Ottawa, Canada.*

A study has been made of the effects of varying the number of doses of stilbestrol over different periods of time on the reproductive organs of the male rat, and it was found that increasing the number of injections has the same effect as giving a larger dose over a shorter period of time, probably by keeping a sustained level of stilbestrol in the body. The effect of relatively small doses over a long period of time was confirmed by giving microgram doses (1, 5 and 25) orally over a period of 180 days. During this time no tolerance to stilbestrol was demonstrated. A linear relationship was obtained when the logarithm of the dose of stilbestrol administered (0.0125–5.0 mg.) was plotted against the percentage decrease in testes weight. This relationship was also found when the log of the dose was plotted against the percentage decrease in body weight although this line had a much more gradual slope. When the administration of stilbestrol was discontinued, the seminal vesicles, coagulating glands and ventral prostates regained their weight by the third to fourth week but the testes remained below the control values until the sixth to ninth week. A study has also been undertaken to ascertain the effect of stilbestrol on organ weights in the hypophysectomized male rat and the findings will be discussed.

Anticoagulant activity of dextran sulfate esters. HOMER E. STAVELY, PHILIP J. BAKER JR. AND H. G. PAYNE (introduced by ALFRED E. WILHELMI). *Pharmaceutical and Chemical Research Divs., Commercial Solvents Corp., Terre Haute, Ind.*

Since the discovery by Howell (*Bull. Johns Hopkins Hosp.* 42: 199, 1928) that heparin contains sulfate groups (subsequently shown to be attached by an ester linkage to the carbohydrate moiety), a large number of polysaccharide sulfate esters have been prepared and tested for anticoagulant activity. Many of these possess considerable activity but have been found to be too toxic for clinical use. In 1945 Grönwall and coworkers prepared sulfuric acid esters of partially degraded dextran of varying molecular weight and concluded that all were too toxic for human use. In connection with our dextran research program we have prepared and tested a large number of dextran sulfate esters in which the average molecular weight, sulfate groups per anhydroglucose unit, and degree of branching has varied. *In vitro* anticoagulant activity was virtually independent of the molecular weight above 10,000.

No significant difference in activity was found between the limits of 1 and 2 sulfate groups per anhydroglucose unit; the trisulfate esters could not be readily prepared. Acute toxicities, however, were roughly proportional to the molecular weight. Esters prepared from dextran having molecular weights of 10,000–30,000 were significantly less toxic to mice than those having higher molecular weights. This conclusion is in accord with the work of Walton (*Proc. Royal Soc. Med.* 44: 563, 1951). The best preparations were about $\frac{1}{8}$ as active as sodium heparin (Lilly) when tested *in vitro*, but were $\frac{1}{3}$–$\frac{1}{2}$ as active when tested in rabbits.